Annex 10 A

TENTATIVE INSTRUCTIONS FOR DETERMINING THE SUSCEPTIBILITY OR RESISTANCE OF BLACKFLY LARVAE TO INSECTICIDES

1. Introduction

(*a*) This method measures the susceptibility levels of a population of blackfly larvae to a given insecticide. The technique is designed to detect the emergence of an insecticide-resistant strain if and when it appears. For this purpose, it is necessary (i) to establish the susceptibility levels of normal blackflies of the population concerned, and (ii) to make subsequent routine checks of the susceptibility levels at periodic intervals. This test is devised specifically to detect physiological resistance. It is stressed that this test is not designed to evaluate the effectiveness of deposits of insecticides in the field ; this must be ascertained by the use of other entomological techniques.

(*b*) *Establishing the base-line.* Batches of blackfly larvae are exposed to different concentrations of insecticide to determine the kills obtained at each level. It is suggested that a preliminary test be made at each concentration in the complete range provided. This will indicate the general levels of susceptibility ; further tests should then be made on this basis. A series of five concentrations should be chosen, some of which give partial mortalities (i.e., at least one of them should give a complete kill and one less than 50% mortality) ; tests at these concentrations should be repeated four times with samples from the same population of blackfly larvae.

(*c*) *Subsequent routine checks.* The concentration double the lowest concentration that has consistently given complete kills in the four successive tests is chosen for the routine checks. These should be made periodically with 2-5 replicates. The occasional appearance of survivors in such checks may be due to normal variation. However, the regular occurrence of survivors (e.g., on three successive occasions) constitutes an alert signal calling for further investigations. Such investigations will include four tests at each of the concentrations used in establishing the original base-line. This would indicate the presence of physiological resistance and the approximate proportion of resistant blackfly larvae in the population.

(*d*) *Condition of blackfly larvae.* Last-stage larvae should be used as they can be identified from pupal respiratory filaments.

(*e*) *Conditions of test.* Tests should be done in a place free from insecticidal contamination. The larvae are exposed at a temperature between 20° and 30°C.

2. Composition of the test kit

(*a*) Five concentrations of each insecticide—DDT and fenthion—in the form of 50-ml standard solutions in ethanol; the final concentrations indicated on the labels are those obtained when 1 ml is diluted with 249 ml of water, namely, 0.004, 0.02, 0.1, 0.5 and 2.5 p.p.m. Fifty ml of ethanol are also provided for the control.

(*b*) Three 1-ml pipettes, one for each insecticide and one for the ethanol. Each pipette is equipped with a small rubber suction bulb for drawing up test solutions.

The user is expected to supply his own collecting and test vessels, as well as the air compressor.

3. Collection and transport of larvae

Larvae are taken from natural breeding places by collecting the submerged and trailing grasses and other vegetation to which they are attached. Although several species of blackfly may occur in the same stream it is sometimes possible, with the help of local knowledge of the breeding site, to collect almost pure samples of a particular species, such as *Simulium damnosum*. The composition of the sample collected can be checked more accurately by later examination of pupae in the laboratory. For transport to the laboratory, the vegetation with attached larvae is kept wet in a bucket or plastic bag, but must not be immersed in water. In this way, the larvae will keep in good condition for two or three hours, and should therefore be transferred from the field to the laboratory within this period. For a complete test with one insecticide 300-600 individuals of the same species must be collected.

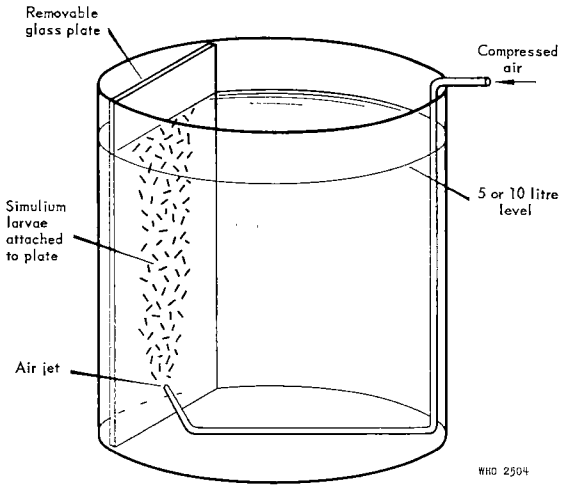
4. Procedure

First alternative.

(*a*) Use 12 glass cylindrical jars of about 5 litres capacity (the possibility of using smaller containers should be explored). Six of the glass jars are used for separating larvae from the vegetation brought in from the natural breeding place and for the recovery period. Six other jars are used for exposure of larvae to insecticides.

(*b*) For the extraction of larvae from the field material, six jars are filled with clean stream or river water and a vertical glass plate (approximately 10 cm × 30 cm) is inserted on one side of each jar. By means of an electric pump, diaphragm pump or battery-operated aerator, a steady stream of compressed air is directed against the foot of the glass plate through a glass tube drawn out into a fine point, thus producing a stream of air-bubbles rising vertically to the top of the glass plate (see accompanying figure).

TEST CHAMBER FOR SIMULIUM LARVAE



Grass and vegetation with attached larvae are immersed in these jars, and in the course of a few hours larvae are induced to leave the vegetation and congregate on the glass plate along the line of agitation caused by the stream of bubbles. When sufficient larvae are attached in this way, those early stages not required for the test can be picked off by forceps and discarded. The same method can be used to reduce the number of fourth-instar larvae to a standard sample of 25 to 50.

(*c*) While this extraction of larvae from vegetation is proceeding, the second set of six containers is filled to the 5-litre mark (or less if indicated) and the required amount of insecticide solution is added to five of them to produce a series of concentrations from 0.01 to 0.25 p.p.m. (see table), mixing being facilitated by a stream of bubbles from an aerator. In the sixth jar, used as a control, an equal amount of solvent only is placed.

(*d*) The following table indicates the procedure used to obtain the necessary concentrations by pipetting out the required quantity of solution into 5 litres of water:

Insecticide concentration suggested for the test (p.p.m.)	Final concentration indicated on bottle of standard solution (p.p.m.)	Number of ml to be added to 5 litres of water
0.25	2.5	2
0.1	0.5	4
0.05	0.5	2
0.02	0.1	4
0.01	0.1	2

(*e*) Each of the glass plates in the first series of jars containing the standard number and samples of attached larvae is quickly transferred to the corresponding position in one of the insecticide testing jars and a continuous stream of air-bubbles immediately directed on to it. If the transfer is done quickly, only a few larvae will be detached in the process and these will normally find their way back to the plate again.

(*f*) After 30 minutes' exposure, the plates with attached larvae are quickly removed from the testing jars and placed back in their original positions in the first series of jars (which in the interval have been emptied and refilled with clean river water). Again, rapid transfer of the glass plates and their adjustment into a position where the jet of the air-bubbles impinges directly on them will ensure minimum loss of larvae through detachment. If during the standard exposure period to insecticide the larvae show any signs of becoming unable to retain a hold on the plate and fall to the bottom of the jar, this probably indicates that the insecticide concentration used is too high for the test series.

(*g*) The larvae are maintained in the holding-jars with a continuous stream of air-bubbles directed on to the plate throughout the following 24- or 48-hour period, during which time it is not normally necessary to change the water. Continue from step (*h*) of second alternative procedure.

Second alternative :

(*a*) Introduce into twelve enamel or glass trays about the necessary quantity of stream water to produce a water depth of at least 1 cm but not exceeding 1.5 cm.

(*b*) Fit each enamel tray with a drawn-out pipette through which compressed air is injected into the water along one side of the tray to keep the water aerated and running. The air must be supplied by an aquarium pump or other available air compressor.

(*c*) Introduce into each tray 25 to 50 last-instar larvae collected from the vegetation to which they were attached by gentle handling with forceps.

(*d*) Prepare the test concentrations by pipetting 1 ml of the standard insecticide solutions under the surface of 1250 ml of distilled water in separate glass or enamel vessels and stirring vigorously for 30 seconds with the pipette or with a glass rod. The final concentrations so obtained will be one-fifth of those indicated on the labels of the bottles used (e.g., the bottle labelled 0.5 p.p.m. will give a concentration of 0.1 p.p.m., since 1 ml will have been diluted to 1250 ml instead of to 250 ml). The control should be prepared by the addition of 1 ml of ethanol in the container.

(*e*) When the larvae have attached themselves to the trays, remove the water gently and replace it by an equal quantity of diluted insecticide solution. Two replicates should be done at each concentration, and two control replicates.

(*f*) After 30 minutes' exposure, remove the insecticide solution gently, rinse gently with river water, and refill the trays with the same amount of river water as before. If some larvae have become detached, rinse them gently in a strainer and replace them in the tray.

(*g*) Change the water in each tray every 4-6 hours during the recovery period.

(*h*) After a period of 24 hours, make mortality counts. In recording the percentage for each concentration, the moribund and dead larvae in both replicates should be combined. Larvae are considered dead if they cannot be induced to move when probed with a needle. Moribund larvae may show discoloration, unnatural positions, tremors, rigor, or inability to stay attached.

(*i*) Discard the larvae that have pupated during the test. The identification of larvae used should be checked on dead and alive larvae of each batch at the completion of the test. If during the test more than 10% of the control larvae pupate, the test should be discarded. Tests with a control mortality of 20% or more are unsatisfactory and should be repeated.

5. General remarks

(*a*) The accuracy of the concentrations provided will be affected if the alcohol is allowed to evaporate from the standard solutions. The bottles should therefore be tightly stoppered after use. The contents should no longer be used when they have decreased below 5 ml; fresh standard solutions should then be obtained from WHO.

(*b*) Test vessels should be carefully cleaned after use to remove traces of insecticide. They should be thoroughly rinsed, scrubbed with detergent and water, or cleaned with potassium dichromate solution, and rinsed again. Pipettes should be thoroughly cleaned with acetone or alcohol.

6. Results

(*a*) The user may desire to construct the dosage-mortality regression line from the results obtained in the quadruplicate tests at the chosen concentration and, in many cases, from those of the single preliminary test as well. For this purpose, the results should be plotted on the logarithmic-probability paper provided. The regression line may be fitted by eye, and the LC_{50} or LC_{90}[1] read from the graph. The regression line should not be extended (extrapolated) beyond the highest mortality obtained.

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[1] The LC_{50} and LC_{90} represent respectively the insecticide concentrations at which 50% and 90% of the specimens are killed.

For more accurate methods of computing the LC_{50}, the worker is referred to the methods described by Swaroop [1] and Litchfield & Wilcoxon.[2]

(*b*) In tests where the control mortality is between 5% and 20%, the percentage mortalities should be corrected by Abbott's formula :

$$\frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100$$

7. Interpretation of results

See Annex 14 : *Criteria and meaning of tests for determining the susceptibility of insects to insecticides.*

8. Distribution of reports

One copy of each report form as completed should be sent to the World Health Organization, Vector Control Unit, Geneva, Switzerland.

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[1] Swaroop, S. (1958) *Statistical methodology in malaria work*, unpublished mimeographed document WHO/Mal/240

[2] Litchfield, J. T., jr & Wilcoxon, F. (1949) *J. Pharmacol. exp. Ther.*, **96**, 99

Specimen Report Form

WHO TEST FOR INSECTICIDE-RESISTANCE IN BLACKFLY LARVAE

Date .....

Insecticide : DDT/fenthion/other [1] ..... *Species :* .....

1. Investigator: .....
2. Country : ..... 3. Province : ..... 4. Locality : .....
5. History of insecticide treatment (including agriculture) : .....

.....

6. Condition of larvae : Instar ..... reared/collected/other [1] .....
7. Results of test (abbreviations : "M" — moribund ; "D" — dead)

Tests	Replicate 1			Replicate 2			Replicate 3			Replicate 4			Totals (for comparable tests only)		
Date of test															
Temperature during test															
Insecticide concentration (p.p.m.)	M & D	Total	Mort. (%) corr.[2]	M & D	Total	Mort. (%) corr.[2]	M & D	Total	Mort. (%) corr.[2]	M & D	Total	Mort. (%) corr.[2]	M & D	Total	Mort. (%) corr.[2]
Control 1															

[1] Cross out what does not apply.

[2] Correct by applying Abbott's formula if control mortality is between 5% and 20% (see instructions).

Remarks : .....

Signature of investigator : .....

One copy of this form to be sent on completion to : World Health Organization, Vector Control Unit, Division of Environmental Health, Geneva, Switzerland.

Annex 10 B

TENTATIVE INSTRUCTIONS FOR DETERMINING THE SUSCEPTIBILITY OR RESISTANCE OF ADULT BLACKFLIES TO INSECTICIDES

1. Introduction

(*a*) This method measures the susceptibility levels of a population of adult blackflies to a given insecticide. The technique is designed to detect the emergence of an insecticide-resistant strain if and when it appears. For this purpose, it is necessary (i) to establish the susceptibility levels of normal blackflies of the population concerned, and (ii) to make subsequent routine checks of the susceptibility levels at periodic intervals. The test is specifically devised to detect physiological resistance. It is stressed that this test is not designed to evaluate the effectiveness of deposits of insecticides in the field ; for this purpose other entomological techniques must be used.

(*b*) *Establishing the base-line*. Batches of blackflies are exposed to different concentrations of insecticide and the kills obtained at each level are determined. It is suggested that a preliminary test be made at each concentration in the complete range provided, using the standard exposure of one hour. This will indicate the general level of susceptibility ; further tests should then be made on this basis. A series of four concentrations should be chosen, some of which will give partial mortalities (i.e., at least one of them should give a complete kill, and one should give less than 50% mortality). Tests at these concentrations should be repeated four times with samples from the same population of blackflies. To reveal the full range of natural variation, this set of tests should be made at several locations and at different seasons, so far as this is practicable. Even if a true base-line (i.e., for an untreated population) cannot be established owing to previous applications of insecticides for any purpose, the susceptibility level of the vector species should be determined immediately.

(*c*) *Subsequent routine checks*. Double the lowest concentration that has consistently given complete kills in the four successive tests is chosen for the routine checks. These should be made periodically with five replicates ; when blackflies are scarce, a minimum of two replicates is permissible. The occasional appearance of survivors in such checks may be due to normal variation. However, the regular occurrence of survivors (e.g., on three successive occasions) constitutes an alert signal calling for further investigation. Such investigations will include four tests at each of the concentrations used in establishing the original base-line. To validate the

results, the investigator must make every effort to collect sufficient specimens for providing at least 15 blackflies per tube.

(*d*) *Condition of blackflies.* Females should be used exclusively. It is recommended that the blackflies selected for test be females that have recently fed and show the presence of a blood meal. If blackflies are scarce, it is permissible to use a mixture of fed and unfed females, provided the proportion of each is recorded. Blackflies may be collected from human or animal baits. In instances where it is not possible to collect a sufficient number of adult blackflies for testing, specimens may sometimes be obtained by collecting the immature stages and rearing adults from them. In some circumstances, females that have not had a blood meal may be used exclusively, e.g., those recently emerged from a collection of pupae.

(*e*) *Conditions of test.* The experiments should be done indoors, if possible, in buildings free from insecticidal contamination and extremes of temperature, humidity and illumination, and away from draughts. Where possible, subsequent comparison tests should be made under similar conditions of temperature and humidity. Transportation of insects to a base laboratory often results in mortality from causes other than the insecticide; this will be evident as high mortality in the controls.

2. Composition of test kit

(*a*) Twenty plastic tubes, 125 mm long by 44 mm in diameter; 8 of these (with red dot) are used for exposing the blackflies to the insecticide; 2 (with green dot) are used for the control exposure without insecticide; 10 (with green dot) are used as holding-tubes for pre-test sorting and post-exposure observation. Each tube is fitted at one end with a 16-mesh screen. In order to identify the concentrations used with them, the red exposure tubes should be numbered 1 to 8, the green control exposure tubes 9 and 10, and the holding-tubes 1*a* to 10*a*.

(*b*) Ten slide-units, each with a screw-cap on either side and provided with a 20-mm filling hole.

(*c*) Five packages of papers impregnated with DDT (*p*,*p*′ isomer) in mineral oil (Risella 17, Shell) at concentrations of 0.0312%, 0.0625%, 0.125%, 0.25%, and 0.5% respectively; and one package treated with oil only. Seven packages of papers impregnated with dieldrin (purified HEOD) in mineral oil at concentrations of 0.005%, 0.01%, 0.025%, 0.05%, 0.1%, and 0.2% and one package treated with oil only.[1]

(*d*) Sheets of clean paper (12×15 cm) for lining the holding-tubes.

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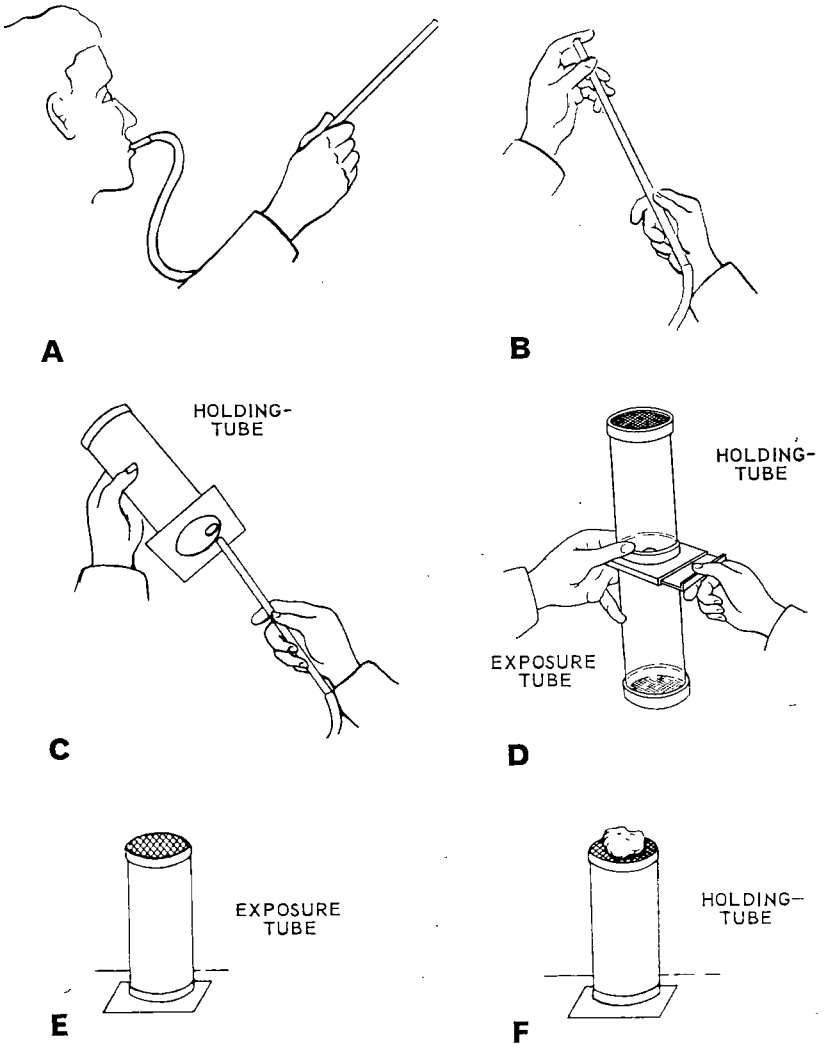
[1] Each package contains eight papers.

FIG. 1. METHOD FOR DETERMINING THE SUSCEPTIBILITY OR RESISTANCE OF ADULT BLACKFLIES TO INSECTICIDES



(*e*) Twenty spring-wire clips to hold the papers in position against the walls of the tubes. The 12 silver clips should be used only for the holding-tubes and the control exposure tubes; the 8 copper clips should be used only for the insecticide exposure tubes.

(*f*) Two glass aspirator tubes, 12 mm in internal diameter, together with 60 cm of tubing.

(*g*) One roll of self-adhesive plastic tape.

(*h*) Instruction sheets and a set of report forms; three sheets of log-probability paper for plotting regression lines.

3. Procedure

(*a*) First a preliminary test is performed with the complete range of concentrations. Into each of the holding-tubes (6 for DDT; 7 for dieldrin), insert a piece of clean white paper rolled into a cylinder to line the wall and fasten it in position with a spring-wire clip (silver). Attach the slides to the tubes.

(*b*) Collect approximately 200 female blackflies with the aspirator provided (Fig. 1, A). Damage resulting from careless handling of blackflies during collecting may produce misleadingly high mortalities. Blackflies should be collected in lots of not more than 5 (Fig. 1, B) and gently transferred to the holding-tubes through the filling-hole in each slide (Fig. 1, C) to give 15 to 25 per tube. Any departure from these figures may impair the reliability of the results.

(*c*) A pre-test holding period may be necessary to guard against including damaged specimens in the test. For this purpose, the holding-tubes are set upright, screen end up, for one hour or more. At the end of this time, the damaged insects are removed.

(*d*) Into each of the exposure tubes (6 for DDT, 7 for dieldrin) introduce a sheet of impregnated paper, rolled into a cylinder to line the wall, and fasten it in position with an appropriate spring-wire clip. One paper impregnated with each of the concentrations of insecticide provided should be used, and one control paper impregnated with oil alone.

(*e*) Introduce the blackflies into the exposure tube by attaching it to the vacant screw-top in the slide (Fig. 1, D). The slide should be pulled out to a point beyond the filling hole so that no part of it occludes the tube openings; the blackflies are then blown gently down into the exposure tube. (If necessary, the small safety knob on the slide may be filed down to facilitate this operation.) Close the slide. Detach the holding-tube and set it aside.

(*f*) Leave the exposure tubes standing upright with screen end up for one hour (Fig. 1, E) under conditions of moderate, diffuse illumination.

(*g*) At the end of the exposure period, transfer the blackflies to the holding-tubes by reversing the process described under (*e*). When some blackflies have been knocked down in the course of an exposure, the exposure tubes should be held horizontally and tapped to dislodge the insects from the slide before withdrawing it. Attach the holding-tube, open the slide, and gently blow the blackflies into the holding-tube ; close the slide and remove the exposure tube. Then set the holding-tube so that it stands on the slide and place a pad of wet cotton-wool on the screen (Fig. 1, F). Cardboard cartons or cups or other suitable containers may be used instead of the holding-tubes, provided that they are used consistently.

(*h*) Keep the holding-tubes for 24 hours in a secluded, shaded place, where the temperature does not exceed 30°C. Wherever feasible, the maximum and minimum temperature of the room during the 24 hours should be recorded. If necessary, the containers should be protected from ants by placing them on a platform standing in a pan of water. If conditions are very hot and dry, a moist chamber may be prepared by suspending damp towelling in a container.

(*i*) Mortality counts are made after 24 hours. Remove the dead blackflies by gently detaching the slide and cautiously moving the tube aside. Affected specimens that are unable to walk are to be counted as dead. As an aid to counting the living specimens, they may be stunned by a sharp jerk of the tube or stupefied by chloroform. The results should be recorded on the forms provided. Copies of completed forms should be distributed in accordance with the instructions (section 7).

(*j*) After the preliminary test has been performed with the complete range of concentrations, tests should be carried out with the chosen series of four concentrations. Four replicates, including the preliminary test where appropriate, should be performed at each of these chosen concentrations. The 20 tubes provided in the kit are sufficient for one series of two replicates at each of the four chosen concentrations, together with two controls.

(*k*) Tests with a control mortality in excess of 20% are unsatisfactory and should be repeated.

(*l*) When the test has been repeated four times with the same population of blackflies, adequate data should be available for constructing a base-line of susceptibility as described in section 5.

4. General remarks

(*a*) Each impregnated paper may be used up to 20 times, and up to three weeks after removal from the package, provided all possible precautions are taken against evaporation of the oil. To this end, the papers should be left in the tubes, with the open end well wrapped, and placed

in the kit box, which in turn should be kept in a cool place. No paper should be used more than three weeks after removal from the package.

(*b*) After an impregnated paper has been removed, the package should be resealed carefully with the plastic tape provided. The packages should be kept in a cool place, but not in a refrigerator, as too low a temperature may cause crystallization in the higher insecticidal concentrations. Prolonged storage at high temperatures should be avoided. Papers should not be used after the expiry date shown on the box. The period of three years from the date of impregnation presupposes that the packages are kept sealed at all times.

(*c*) If the species of blackflies concerned is exceptionally insensitive, the exposure period may be increased to 2, 4, or 8 hours. In all cases, the 1-hour exposure period should be used first and the results recorded.

5. Results

(*a*) The user may desire to construct the dosage-mortality regression line from the results obtained in the quadruplicate tests at the chosen concentrations and, in many cases, from those of the single preliminary test as well. For this purpose, the results should be plotted on the logarithmic-probability paper provided. The regression line may be fitted by eye, and the LC_{50} or LC_{90}[1] read from the graph. The regression line should not be extended (extrapolated) beyond the highest mortality obtained. For more accurate methods of computing the LC_{50}, the worker is referred to the methods described by Swaroop [2] and Litchfield & Wilcoxon.[3]

(*b*) In tests where the control mortality is between 5% and 20%, the percentage mortalities should be corrected by Abbott's formula:

$$\frac{\%\text{ test mortality} - \%\text{ control mortality}}{100 - \%\text{ control mortality}} \times 100$$

6. Interpretation of results

See Annex 14: *Criteria and meaning of tests for determining the susceptibility of insects to insecticides.*

7. Distribution of reports

One copy of each report form as completed should be sent to the World Health Organization, Vector Control Unit, Geneva, Switzerland.

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[1] The LC_{50} and LC_{90} represent respectively the insecticide concentrations at which 50% and 90% of the specimens are killed.

[2] Swaroop, S. (1958) *Statistical methodology in malaria work*, unpublished mimeographed document WHO/Mal/240

[3] Litchfield, J. T., jr, & Wilcoxon, F. (1949) *J. Pharmacol. exp. Ther.*, **96**, 99

Specimen Report Form

WHO TEST FOR INSECTICIDE-RESISTANCE IN ADULT BLACKFLIES

Date _____ Record No. _____

Insecticide : DDT/dieldrin/other [1] _____ *Species* : _____

1. Investigator : _____ 2. Country : _____
3. Province : _____ 4. Locality : _____
5. History of insecticide treatment (including agriculture) : _____

6. Condition of blackflies : blood-fed/gravid/unfed/sugar-fed/males [1] 7. Where collected : shelters sprayed/unsprayed/outdoors/biting/bred out [1]
8. Type of test on population : first time/routine check/complete retest [1] 9. Exposure period (minutes) : _____

Tests	Preliminary			Replicate 1			Replicate 2			Replicate 3			Totals (for comparable tests only)		
Date of test															
Temperature during exposure period															
Humidity during exposure period (%)															
Temperature during 24-hr holding period (°C)	Max. Min.			Max. Min.			Max. Min.			Max. Min.					
Insecticide concentration (%)	Dead	Total	Mort. (%) corr.[2]	Dead	Total	Mort. (%) corr.[2]	Dead	Total	Mort. (%) corr.[2]	Dead	Total	Mort. (%) corr.[2]	Dead	Total	Mort. (%) corr.[2]
Control (oil alone)															

[1] Cross out what does not apply. [2] Correct by applying Abbott's formula if control mortality is between 5% and 20% (see instructions).

Remarks : _____

Interpretation of results (optional) : _____

Signature of investigator : _____

One copy of this form to be sent on completion to : World Health Organization, Vector Control Unit, Division of Environmental Health, Geneva, Switzerland.

Annex 11

TENTATIVE INSTRUCTIONS FOR DETERMINING THE SUSCEPTIBILITY OR RESISTANCE OF ADULT TICKS TO INSECTICIDES

1. Introduction

(*a*) This method may be used to measure susceptibility levels of a population of adult ticks to a given insecticide. The technique is designed to detect the emergence of an insecticide-resistant strain if and when it appears. For this purpose, it is necessary (i) to establish the susceptibility levels of normal adult ticks of the population concerned, and (ii) to make subsequent routine checks of the susceptibility levels at periodic intervals. The test is devised specifically to detect physiological resistance.

(*b*) *Establishing the base-line*. Batches of adult ticks are topically treated with different concentrations of insecticides to determine the kills obtained at each level. It is suggested that a preliminary test be made at each concentration in the complete range provided for each species at the standard dosage suggested. This will indicate the general level of susceptibility; further tests should then be made on this basis. A series of four concentrations should be chosen some of which will give partial mortalities (i.e., at least one of them should give a complete kill and one less than 50% mortality); tests at these concentrations should be repeated four times with samples from the same population. To reveal the full range of natural variation, this test should be made at several locations and at different seasons, so far as this is practicable.

(*c*) *Subsequent routine checks*. The lowest concentration that has consistently given complete kills in the four successive tests is chosen for the routine checks. These should be made periodically with two to five replicates. The occasional appearance of survivors in each check may be due to normal variation. However, the regular occurrence of survivors (e.g., on three successive occasions) constitutes an alert signal calling for further investigations. Such investigations will include four tests at each of the concentrations used in establishing the original base-line. This would indicate the presence of physiological resistance and the approximate proportion of resistant individuals in a population.

(*d*) *Sex of test insects*. Adults of either sex may be used.

(*e*) *Condition of the test*. Tests should be done in a room free from insecticidal contamination. The insects are treated and held at a temperature between 20° and 30°C and at a relative humidity above 25%.

2. Composition of the test kit

(*a*) Five glass microcapillary tubes for delivery of 0.38-microlitre doses.

(*b*) Five glass holding-tubes, 14 cm in length and 7 mm in external diameter; the microcapillary is fixed at one end by means of a plug 3 mm long, with 0.5-1.0 mm of the capillary tube projecting from one end of the plug; the other end is connected by rubber tubing to a glass mouthpiece or a rubber bulb.

(*c*) Five reservoir tubes.

(*d*) Five dip tubes with a rubber ring.

(*e*) Five metal clips for holding the reservoir tube.

(*f*) Five 50-ml bottles containing the following concentrations of insecticides in methyl ethyl ketone:

DDT (*p*,*p*'-isomer)	10%
Dieldrin (purified HEOD)	5%
Malathion	10%
Fenthion	5%
Control	methyl ethyl ketone.

(*g*) Twenty-four glass test-tubes, 15 cm long by 15 mm internal diameter.

(*h*) Thirty caps of coarse mesh to fit over the tubes.

(*i*) Fifty rubber bands.

(*j*) Collecting apparatus—two pairs of forceps.

(*k*) Twenty-five holding containers for insects (plastic tubes with coarse screen).

(*l*) Instruction sheets and set of report forms plus three sheets of log-probability paper for plotting regression lines.

3. Method of test

(*a*) Hard ticks are collected from animals and soft ticks are collected from dust in human dwellings and litter from animal houses. The required concentration of insecticide is then applied topically on the dorsal side. The arthropods are held in the holding-containers and the percentage mortality recorded after 24 hours. Insects unable to move are counted as dead.

(*b*) After the preliminary test has been performed with the complete range of concentrations, tests should be carried out with the chosen series of four concentrations. Three replicates should be performed at each of the chosen concentrations giving partial and complete mortality.

(*c*) Tests with a control mortality in excess of 20% are unsatisfactory and should be repeated.

(*d*) When the tests have been repeated four times with the same populations of insects, adequate data should be available for constructing a base-line of susceptibility, as described in section 4.

(*e*) After the required quantity of insecticide solution has been removed, the stock bottles should be carefully closed, and stored in a cool place.

4. Results

(*a*) The user may desire to construct the dosage-mortality regression line from the results obtained in the quadruplicate tests at the chosen concentrations and in many cases from those of the single preliminary test as well. For this purpose the results should be plotted on the logarithmic-probability paper provided. The regression line may be fitted by eye and the LC_{50} or LC_{90}[1] read from the graph. The regression line should not be extended (extrapolated) beyond the highest mortality obtained. For more accurate methods of computing the LC_{50}, the worker is referred to the methods described by Swaroop [2] and Litchfield & Wilcoxon.[3]

(*b*) In tests where the control mortality is between 5% and 20%, the percentage mortality should be corrected by Abbott's formula:

$$\frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100$$

5. Interpretation of results

See Annex 14: *Criteria and meaning of tests for determining the susceptibility of insects to insecticides.*

6. Distribution of reports

One copy of each report form as completed should be sent to World Health Organization, Vector Control Unit, Geneva, Switzerland.

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[1] The LC_{50} and LC_{90} represent respectively the insecticide concentrations at which 50% and 90% of the specimens are killed.

[2] Swaroop, S. (1958) *Statistical methodology in malaria work*, unpublished mimeographed document WHO/Mal/240

[3] Litchfield, J. T., jr & Wilcoxon, F. (1949) *J. Pharmacol. exp. Ther.* **96**, 99

Specimen Report Form

WHO TEST FOR INSECTICIDE-RESISTANCE IN ADULT TICKS

Date: .....

1. Investigator: ..... 2. Species: .....
3. Country: ..... 4. Province: ..... 5. Locality: .....
6. History of insecticide treatment (including agriculture): .....
7. Type of test on population: first time/routine check/complete retest [1]

Tests	Preliminary			Replicate 1			Replicate 2			Replicate 3			Totals (for comparable tests only)		
Date of test															
Temperature during exposure period															
Humidity during exposure period															
Temperature during holding period	Max. Min.			Max. Min.			Max. Min.			Max. Min.					
Insecticide conc. (%) DDT/dieldrin/other [1]	Dead	Total	Mort. (%) corr.[2]	Dead	Total	Mort. (%) corr.[2]	Dead	Total	Mort. (%) corr.[2]	Dead	Total	Mort. (%) corr.[2]	Dead	Total	Mort. (%) corr.[2]
Control															

[1] Cross out what does not apply.
[2] Correct by applying Abbott's formula if control mortality is between 5% and 20% (see instructions).

Remarks: ..... Signature of investigator: .....

One copy of this form to be sent on completion to: World Health Organization, Vector Control Unit, Division of Environmental Health, Geneva, Switzerland.

Annex 12

TENTATIVE INSTRUCTIONS FOR DETERMINING THE SUSCEPTIBILITY OR RESISTANCE OF COCKROACHES TO INSECTICIDES

1. Introduction

(*a*) This method measures the susceptibility levels of a population of cockroaches to a given insecticide. The technique is designed to detect the emergence of an insecticide-resistant strain if and when it appears. For this purpose it is necessary (*i*) to establish the susceptibility levels of normal cockroaches of the population concerned, and (ii) to make subsequent routine checks of the susceptibility levels at periodic intervals. This test is devised specifically to detect physiological resistance.

(*b*) *Establishing the base-line.* Batches of cockroaches are exposed to standard insecticide residues in glass jars and observed to determine their rate of knockdown. From the results, the times necessary for 50% and 100% knockdown (LT_{50} and LT_{100}) can be determined. These values should be determined for at least five batches of approximately 20 insects. To reveal the full range of natural variation this test should be made at several locations and in different seasons, so far as this is practicable.

(*c*) *Subsequent routine checks.* These should be made periodically with 2-5 replicates. The occurrence of resistance will be indicated by a pronounced change in the rate of knockdown, including a significant percentage of cockroaches unaffected at the end of the exposure period.

(*d*) *Condition of cockroaches.* It is preferable to use adult males. If it is not feasible to obtain enough males, information on susceptibility can be obtained by using females and nymphs.

(*e*) *Conditions of test.* Tests should be done in a room free from insecticidal contamination. The cockroaches are exposed and held at a temperature between 20° and 30°C and at a relative humidity above 25%. Some uniformity in these conditions can be secured by the use of the kit box.

2. Composition of test kit

(*a*) 50-ml stock solutions of dieldrin (purified HEOD, 0.5%),[1] malathion (1.0%), and diazinon (1.0%).

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[1] Dieldrin is supplied as a non-volatile test insecticide for detecting resistance to compounds of the cyclodiene class, including chlordane.

(*b*) Four bottles of acetone each containing 50 ml.

(*c*) Three 10-ml measuring cylinders.

(*d*) Three 5-ml graduated pipettes.

(*e*) Six 1-pint glass jars.

(*f*) One jar of petrolatum grease.

(*g*) One jar of petrolatum oil.

(*h*) Instruction sheets and set of report forms; three sheets of log-probability paper for plotting regression lines.

3. Method of test

(*a*) Cockroaches should be obtained, as far as possible, from the same area, and kept in a suitable container until required.

(*b*) Add 1 ml of stock solution to 9 ml of acetone and place 2.5 ml of this diluted solution in each of four jars. Roll the jars horizontally so that the solution runs evenly over the bottom and sides until the acetone evaporates. It is convenient to treat the jars in the afternoon and conduct the test the following morning.

(*c*) Smear the inside of the neck of the jars with a thin film of a mixture of equal parts of the two types of petrolatum to prevent the cockroaches from escaping. The petrolatum blends more readily if heated.

(*d*) Introduce 10 cockroaches into each of the jars and record knock-down periodically as indicated on the report form. At each observation the percentage of cockroaches knocked down is recorded until 90-100% are knocked down. If any are still active after 24 hours, a piece of raw potato is provided as food.

4. Results

The user may desire to construct the time-knockdown percentage regression line from the results obtained in the quadruplicate tests at the chosen concentration. For this purpose, the results should be plotted on the logarithmic-probability paper provided. The regression line may be fitted by eye, and the LT_{50} or LT_{90}[1] read from the graph. The regression line should not be extended (extrapolated) beyond the highest knockdown percentage obtained.

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[1] The LT_{50} and LT_{90} represent respectively the times required for 50% and 90% of the specimens to be knocked down.

5. Interpretation of results

See Annex 14 : *Criteria and meaning of tests for determining the susceptibility of insects to insecticides.*

6. Distribution of reports

One copy of each report form as completed should be sent to the World Health Organization, Vector Control Unit, Geneva, Switzerland.

Specimen Report Form

WHO TEST FOR INSECTICIDE-RESISTANCE IN COCKROACHES

Date : .....

1. Investigator : ..... 2. Species : .....
3. Country : ..... 4. Province : ..... 5. Locality : .....
6. History of insecticide treatment (including agriculture) : .....
7. Age and sex of cockroaches : .....
8. Where collected : human habitation/animal shelter/other : .....
9. Type of test on population : first time/routine check [1]
10. Insecticide : malathion/diazinon/dieldrin [1] — concentration ..... %

Tests		Preliminary				Replicate 1			Replicate 2			Replicate 3			
Date of test															
Temperature range during exposure		Max. Min.				Max. Min.			Max. Min.			Max. Min.			
Humidity range during exposure		Max. Min.				Max. Min.			Max. Min.			Max. Min.			
Test No.	No. used	Number knocked down after indicated observation period (hours)													
		0.5	1	1.5	2	2.5	3	3.5	4	5	6	7	24	48	72
Control (acetone alone)															

[1] Cross out what does not apply.

Remarks : ..... Signature of investigator : .....

One copy of this form to be sent on completion to : World Health Organization, Vector Control Unit, Division of Environmental Health, Geneva, Switzerland.

Annex 13

TENTATIVE TEST PROCEDURE FOR DETECTING RESISTANCE TO DICHLORVOS AND OTHER PERSISTENT FUMIGANTS

1. Introduction

(*a*) This method may be used to measure susceptibility levels in populations of mosquitos and other insects to dichlorvos and other persistent fumigants. The technique is designed to detect the emergence of a resistant strain, if and when it appears. For this purpose it is necessary (i) to establish the susceptibility level of normal specimens of the population concerned, and (ii) to make subsequent routine checks at periodic intervals. The test is devised to detect physiological resistance.

(*b*) *Establishing the base line.* Batches of insects are exposed to a standard concentration of vapour and observed to determine their rate of knockdown. From the results the times necessary for 50% and 100% knockdown (LT_{50} and LT_{100}) can be determined. These tests should be based on tests with at least five batches of approximately 20 insects. To reveal the full range of natural variation, this test should be made at several locations and at different seasons, so far as this is practicable.

(*c*) *Subsequent routine checks.* These should be made periodically with 2-5 replicates. The occurrence of resistance will be indicated by a pronounced change in the rate of knockdown, including a significant percentage of insects unaffected at the end of the test period.

(*d*) *Test insects.* In the case of mosquitos and flies, female insects should be used.

(*e*) *Conditions of test.* Tests should be done indoors, avoiding extremes of temperature and humidity, and in diffused lighting.

2. Composition of test kit

(*a*) 2-5-litre glass flasks with stoppers.

(*b*) 6 nylon net cages (3 cm × 8 cm).

(*c*) Reel of cotton thread.

(*d*) Box of filter papers.

(*e*) 50-ml bottle of dichlorvos solution (0.3%) in dioctyl phthalate.

(*f*) Syringe for applying 3-mm³ quantities.

(*g*) Two aspirator tubes.

(*h*) Instruction sheets and set of report forms; three sheets of log-probability paper for plotting regression lines.

3. Method of test

(*a*) Cut a small piece of filter paper approximately 1 cm square and attach it to a length of cotton thread. Apply 3 mm³ of dichlorvos solution to the filter paper and suspend it in the flask, replacing the stopper.

(*b*) Introduce the insects into a nylon cage, close it with a clip and attach it to a piece of cotton thread.

(*c*) Half an hour after closing the flask slip the cage into the flask (quickly removing and replacing the stopper) and suspend it from thread.

(*d*) Observe the condition of the insects at 5-minute intervals and record the knockdown number. When all insects have been knocked down remove the cage from the flask.

(*e*) Transfer the insects to a paper cup covered with nylon mesh and hold for 24 hours to observe possible recoveries.

4. Results

The user may desire to construct the time-knockdown percentage regression line from the results obtained in the quadruplicate tests at the chosen concentration. For this purpose, the results should be plotted on the logarithmic-probability paper provided. The regression line may be fitted by eye, and the LT_{50} or LT_{90}[1] read from the graph. The regression line should not be extended (extrapolated) beyond the highest knockdown percentage obtained.

5. Interpretation of results

See Annex 14: *Criteria and meaning of tests for determining the susceptibility of insects to insecticides.*

6. Distribution of reports

One copy of each report form as completed should be sent to the World Health Organization, Vector Control Unit, Geneva, Switzerland.

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[1] The LT_{50} and LT_{90} represent respectively the times required for 50% and 90% of the specimens to be knocked down.

Specimen Report Form

WHO TEST FOR INSECTICIDE-RESISTANCE TO DICHLORVOS AND OTHER PERSISTENT FUMIGANTS

Date : .....

1. Investigator : ..... 2. Species: .....
3. Country : ..... 4. Province : ..... 5. Locality : .....
6. History of insecticide treatment (including agriculture) : .....
7. Condition of insects : blood fed/unfed [1]
8. Where collected : human habitation/animal shelter/other : .....
9. Type of test on population : first time/routine check/complete retest [1]

Tests		Preliminary				Replicate 1			Replicate 2			Replicate 3			
Date of test															
Temperature range during exposure		Max. Min.				Max. Min.			Max. Min.			Max. Min.			
Humidity range during exposure		Max. Min.				Max. Min.			Max. Min.			Max. Min.			
Test No.	No. used	Number knocked down after indicated observation period (minutes)													
		5	10	15	20	25	30	40	50	60	80	100	120	180	200
Control															

[1] Cross out what does not apply.

Remarks :

Signature of investigator :

One copy of this form to be sent on completion to : World Health Organization, Vector Control Unit, Division of Environmental Health, Geneva, Switzerland.

Annex 14

CRITERIA AND MEANING OF TESTS FOR DETERMINING SUSCEPTIBILITY OR RESISTANCE OF INSECTS TO INSECTICIDES

Use of any standardized method with any insect species involves the assumption that a range of dosage and length of exposure that give a low to complete kill of typical susceptible strains will provide a base-line to which the response of any other population may be compared. The tests are quite arbitrary in the sense that the possible range of dosage is dependent upon the length of exposure and the subsequent period of observation during which mortality is evaluated ; the results would be quite different if these conditions were altered markedly.

In practical use, several difficulties have been encountered, among which may be mentioned :

(*a*) No species in even the most natural conditions is invariable in its response and hence the base-line varies with many conditions, such as season of the year, condition of nutrition, period since a blood meal, stage of egg development, age, etc. The conditions obtaining during a test, e.g., temperature, intensity of light or any other factor influencing the activity of the test insects, will also affect the results.

(*b*) Populations subject to control measures, or any other selection process, often give response lines differing not only in position but also in shape from the characteristic straight log-dosage-probit (ld-p) mortality lines of the reference population. A frequent situation is a marked bending towards higher dosages above a certain mortality (see figure). If the line becomes horizontal and continues to be so up to the highest dosage used, it is obvious that only a portion of the total group can be killed by the procedure and the survivors may be called resistant. In the case of anopheline mosquitos tested for susceptibility to DDT, a number of suggestions have been made to assist the investigator who often works only with the kits furnished by WHO.

The use of different criteria by different investigators is proof that the results are often difficult to interpret. This arises in part from the nature of the ld-p line, which in theory can be straight only if the distribution of sensitivity within a tested group follows the normal distribution. Resistance to insecticides has been shown in an ever-increasing number of species to be genetically determined and the single factor operative in most

cases gives rise to three genotypes whose differences in sensitivity are displayed in the corresponding phenotypes. If two or even three genotypes differing in sensitivity are present in a group of insects, the resulting ld-p line cannot be straight but must have inflexions or horizontal regions corresponding to the percentages of the group contributed by each genotype.

In the case of female anopheline mosquitos, it has been shown by tests with the respective three genotypes that they are well separated in their responses to dieldrin, so that " discriminating dosages " can be used (*a*) to kill all susceptibles leaving hybrids plus resistants, and (*b*) to kill susceptibles plus hybrids leaving only resistants. Thus the make-up of any group can be determined without ambiguity.

In contrast, among several anophelines and many other insects, the susceptible and hybrid genotypes differ less in susceptibility to DDT and the ranges of response to the insecticide overlap. Hence, there can be no truly discriminating dosage, though one that kills all the susceptibles but only a few of the hybrids is sometimes used. The term "recessive" resistance is often used to denote such cases of overlapping susceptibility. In any test including the two genotypes, the ld-p line cannot be straight but must have an inflexion corresponding to the percentage of susceptibles present. The mortality caused by any given dosage will be less than that for the susceptible genotype only. The reduction in mortality is a function of the percentage of hybrids present, but the relation is complex, and the effect varies with the dosage used and the slope of the ld-p line for each genotype.

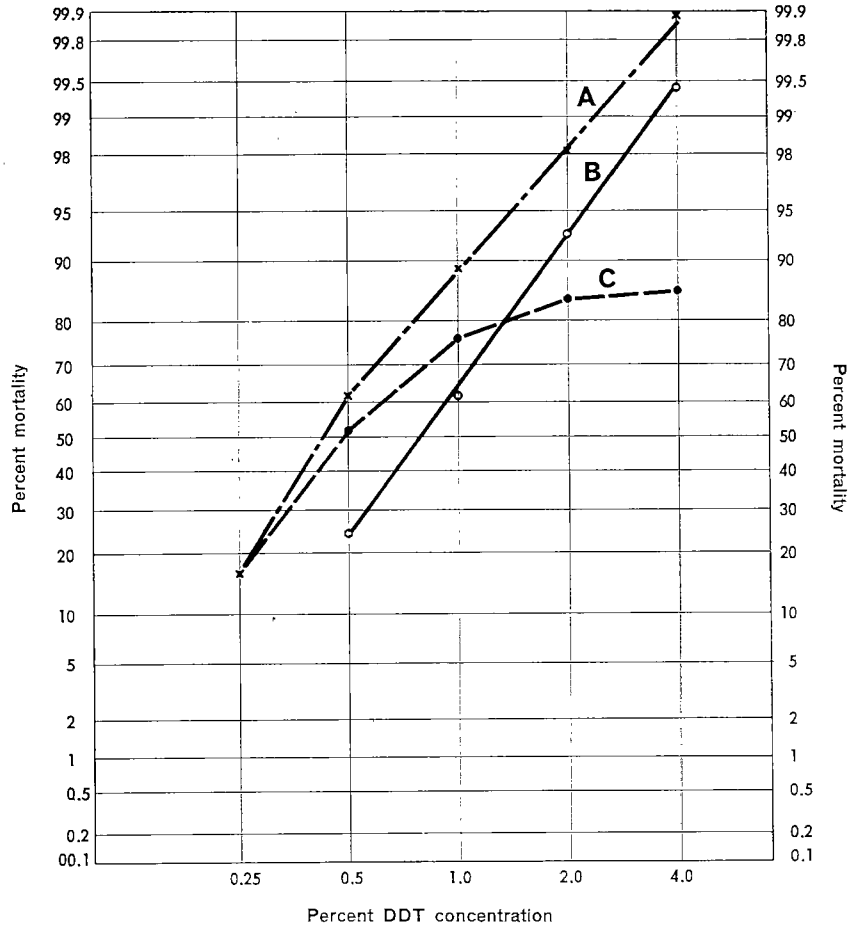
The small decrease in mortality often found with 2% and 4% DDT papers, which has sometimes occasioned much alarm among mosquito control workers, usually indicates only that a small proportion of hybrids is present. With DDT, the hybrids are almost as easily killed as the susceptibles, and a long interval may pass before the appearance of sufficient homozygous resistants to have an adverse effect on control. If these have some biological disadvantage, such as low fertility or short life, they may never have a serious effect upon practical control measures. In the case of dieldrin, however, a small decrease in mortality foreshadows a rapid increase in the proportion of uncontrollable individuals, since the hybrids are almost totally immune to any normal spraying operation.

In the accompanying figure, the sample regression lines show three types of response :

Line A represents the base-line of a susceptible population.

Line B shows, in comparison to line A, a uniform decrease in susceptibility of the population as a whole. Such a decrease, even when statistically significant, could derive merely from seasonal or environmental fluctuations. This shift to the right could be due also to the more permanent development

SAMPLE REGRESSION LINES FOR SUSCEPTIBILITY (A), FOR SEASONAL FLUCTUATION OR FOR VIGOUR TOLERANCE (B), AND FOR PARTIAL RESISTANCE (C) IN THE TEST POPULATION



of vigour tolerance ; [1] it should not be interpreted as the incipient development of true physiological resistance, as this would cause the regression line to shift much further to the right and become characteristically less steep.

Line C is the response of a population in which a proportion of the individuals are resistant to the toxicant. This plateau response is characterized by the failure of the higher concentrations to produce a progressive straight-line increase in mortality.

[1] Hoskins, W. M. (1959) *Mosquito News*, **19**, 52

Occasionally, in areas that have been treated with insecticides and even in untreated localities, populations giving regression lines similar to line C but differing in the proportion of resistant individuals may be found. Where the plateau type of regression line is obtained, the LC_{50} value indicated may be of interest to the worker but it is unreliable for comparison with LC_{50} values derived from regression lines such as A and B.

With other mosquitos and with other kinds of insect the same general principles should guide the interpretation of susceptibility tests. A more detailed discussion of this subject has been undertaken by Hoskins [1] and Davidson.[2]

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[1] Hoskins, W. M. (1960) Use of the dosage-mortality curve in quantitative estimation of insecticide resistance. *Misc. Publ. ent. Soc. Amer.*, **2**, 85

[2] Davidson, G. (1960) The importance of the discriminating dosage in the determination of insecticide resistance in anopheline mosquitoes. *Misc. Publ. ent. Soc. Amer.*, **2**, 93

Annex 15 A

INSTRUCTIONS FOR THE BIO-ASSAY OF INSECTICIDAL DEPOSITS ON WALL SURFACES

1. Introduction

Bio-assays are methods for estimating the potency of a material by means of the response of living matter to it. The main objective of this bio-assay test is to assess the potency of an insecticide deposit for adult mosquitos at various times after application on different surfaces and thus to detect the onset of a definite decline in the toxic effect of the deposit due to aging, sorption or other factors. The test is designed to provide information that may assist in (i) comparing the residual action of different insecticides or insecticide formulations, and (ii) ascertaining whether or not the spraying has been carried out satisfactorily. The method will not measure the amount of insecticide remaining on the wall nor will it by itself measure the overall rate of kill of vectors being achieved during the campaign, since this can only be assessed by other entomological measurements in the area.

It is recommended that where possible such supplementary methods as window-trap collections and survival tests be used in conjunction with the bio-assay test. This is particularly advisable where the bio-assay method is used to determine the time at which an insecticidal deposit on a given surface has lost its potency or to decide on the appropriate insecticide dosage and spacing of spraying cycles for effective vector control. The method is not suitable for measuring the susceptibility or resistance of a population.

2. Conditions of the test

Rigid rules cannot be laid down as to the number and frequency of bio-assay tests which should be conducted in various parts of the world. It must be recognized that each geographic area has its own set of conditions with respect to rainfall, temperature, humidity, surfaces, and vector species. Therefore, the use to which the test is put in a given area and the interpretation of the results will necessarily depend upon local conditions. With these factors in mind, the following guiding principles are suggested :

(*a*) The first tests for the evaluation of any given insecticide should be undertaken within a few days after its application or as soon as the deposit is completely dry. In those instances where a previously sprayed area is due for another application of insecticide and no bio-assay data

are available, it is desirable that bio-assays should be performed beforehand to ascertain the potency of the deposits present.

(*b*) In using this method, it is of great importance to carry out the tests on an adequate scale and at regular intervals. It is necessary to test and to evaluate separately the potency of the insecticide deposit on each main type of resting surface. On a given type of surface, not less than 10 points, variously situated, should be chosen for the bio-assay tests on a given day. They should be distributed in several houses, not more than three points being in any one house. Controls must be run at a rate of at least two controls for every 10 bio-assay tests. The control runs are carried out in a suitable situation away from the sprayed premises and on an unsprayed surface. For this purpose, it is suggested that the investigator carry with him a stock of index cards or similar unsprayed material to be discarded after each test.

(*c*) When the main objective of the investigator is to determine the rate of loss of potency, there will be advantages in using the same points throughout the series of tests. He should therefore mark the points carefully at the time of the first tests and should take particular care to avoid rubbing or in any way impairing the deposit at these points during the performance of the tests. If, on the other hand, different points are selected for the subsequent tests, more information will be gained on the overall potency of the insecticide deposit on the types of surface examined.

(*d*) *Subsequent routine checks*. Following the initial bio-assay, subsequent tests should be made at intervals not exceeding one month. If successive tests are carried out at the same time of day the comparability of the data will be improved.

(*e*) *Choice of test insect*. Wherever possible, the local vector species should be used in the bio-assay. If there is more than one vector species, each should be used separately, and it is preferable to take wild-caught specimens because of their greater hardiness. If a species of mosquito other than the local vector is used, it should preferably be from a laboratory colony. It is recommended that the mosquitos selected for test be females, all of which have recently been fed and show the presence of a blood meal. The susceptibility of the mosquito population to the insecticide being bio-assayed must be established before the first bio-assay (using the standard adult mosquito test kit) and re-checked each time a cycle of testing is done in order to ensure the validity of the bio-assay results. A marked change in susceptibility during the course of the bio-assay testing would invalidate the results.

3. Composition of test kit

(*a*) Twenty-four conical chambers of transparent plastic, 8.5 cm in diameter at the base and 5.5 cm high.

(*b*) Ten hard-glass aspirator tubes, 1 cm in outside diameter (with one end bent so as to facilitate removal of mosquitos from the exposure chamber), together with 60 cm of flexible rubber or plastic tubing. These tubes are suitable for handling mosquitos of ordinary size. For very large species, tubes with an inside diameter of 12 mm should be used. These tubes can be of glass or transparent plastic.

The exposure chambers are best loaded with a straight glass or plastic tube of appropriate diameter; this is not supplied in the test kit.

(*c*) Four 10-metre rolls of adhesive plastic sponge tape, two thick and two thin.

(*d*) One box of upholstery tacks, with large heads.

(*e*) Instruction sheets.

4. Procedure

(*a*) The exposure chamber is fastened to the selected spot on the surface to be tested with the upholstery tacks, or with some appropriate device that will hold the chamber tight against the surface. It is often advantageous to fasten a strip of the plastic sponge tape to the flange of each test chamber and leave it there permanently. Particular care should be taken not to slide the chamber on the surface while it is being attached or removed.

(*b*) At least ten but not more than fifteen mosquitos are collected with a straight "loading" tube and introduced into the chamber by blowing gently, great care being taken that the end of the tube does not touch the test surface. There should be no back pressure; this can be avoided by boring a dozen small holes at the outermost tip of the exposure chamber.

The cage containing the stock of mosquitos to be used in the bio-assay test should never be taken inside a house that has been sprayed with insecticide but should be left on insecticide-free surfaces outside the houses. The cages should be handled with care so as not to contaminate them with insecticide from the hands of the operator.

(*c*) The chamber is left undisturbed for a standard period of time, long enough to cause 100% mortality of the test mosquitos. It has been found that 30 minutes is usually a suitable standard, and it is recommended that this period be chosen when the bio-assays are first started in any area, but a longer period may be used if necessary.

(*d*) At the end of the exposure period, the mosquitos are collected carefully by means of the bent transfer tube introduced through the aperture of the chamber without contacting the test surface and are transferred immediately to the holding containers. Paper cups of half-pint capacity are cheap and convenient, but other types of container may be used.

(*e*) The room temperature and relative humidity are recorded at the beginning and end of each day's testing, and at hourly intervals during the work period.

(*f*) The recovery cages are kept for 24 hours in a secluded, shaded place, where the temperature does not exceed 30°C, if feasible. Maximum and minimum temperatures during the recovery period should be recorded. The humidity may be kept high by use of damp towelling where necessary.

(*g*) The exposure chambers and transfer tubes are carefully washed in detergent after each use, rinsed, and allowed to drain dry. No attempt should be made to reserve certain chambers and tubes for the control mosquitos, and others for the test mosquitos.

(*h*) WARNING. It is of vital importance that the transfer tubes should not become contaminated with insecticide during the course of the day's work. To prevent the undetected occurrence of such contamination, it is essential to use the same transfer tube, first for the test mosquitos and then for the control mosquitos. If a single transfer tube is used for four or five test chambers and then for a control chamber, and a high mortality is observed in the control mosquitos, all results with that transfer tube are considered invalid. The tests should then be repeated using a separate transfer tube for each chamber and for the control. If the transfer tubes should become contaminated with insecticide removed by suction or in any other way from the wall surfaces being tested, it becomes impossible to ascertain how much of the observed mortality is due to the exposure of the mosquitos on the wall surface and how much to insecticide in the transfer tube or holding containers. Even when a separate transfer tube is used for each chamber, there is some risk that insecticide will be transferred from the wall surface to the holding container along with the mosquitos. Should this happen, the mortality of the mosquitos might be inadvertently increased.

5. Results and interpretation

(*a*) After 24 hours, counts of dead and live mosquitos are recorded. It is essential that observed mortalities (in %) be recorded for each individual test. If under the conditions of work, it is found that the control mortality is above 10%, it is recommended that the number of controls in the subsequent series of tests be increased to four for each series of 10 tests. Where control mortalities exceed 20%, the series of tests should be considered unsatisfactory and repeated, if possible.

(*b*) The mortalities on the different points (on one type of surface only) are averaged. Where the control mortality is between 5% and 20%, the average observed mortality is corrected by Abbott's formula :

$$\frac{\%\ \text{test mortality} - \%\ \text{control mortality}}{100 - \%\ \text{control mortality}} \times 100$$

(*c*) Immediately after the average value, the lowest and the highest values should be written in parentheses, thus: " 68% (20%—90%) ", to indicate the range of variation in the results. Wide differences in mortality rates from one point to another may reflect either the unevenness of spraying or a differential in the rate of loss of potency, due to the particular composition of the surface, the soot deposited, the microclimate, or other localized variables. Even if all such factors could be eliminated, there would still remain as a cause of variability the inherent differences in susceptibility of the individual mosquitos used as a tool in the tests.

(*d*) A marked drop in the bio-assay kills in a sprayed area, while not in itself proving that a fresh application of insecticide is necessary, can underline the need for other investigations on the vector species designed to evaluate the continued effectiveness of vector control.

Annex 15 B

INSTRUCTIONS FOR THE BIO-ASSAY OF PERSISTENT FUMIGANTS

Bio-assay of persistent fumigants should be carried out by exposing the caged test insect to the fumigant vapours for a standard period of time within the treated dwelling.

It is preferable to use insectary-bred specimens of the main insect species against which the fumigant has been introduced. If these are not available then wild-caught fed individuals may be used with appropriate controls.

Cages 12.5 cm×6.5 cm×6.5 cm with a wire frame and mosquito netting walls are used. The insects are introduced through a 12.7-cm long sleeve at one end, and the sleeve is then closed by a rubber band. Cages should be suspended within the room to be tested, two at the level of the fumigant dispenser but no closer to it than 3.2 m and two more pinned to the wall at a height of 60 cm from the floor.

In the case of insectary-bred mosquitos, 25 3-day old blood-fed females should be used per cage. If the particular vector concerned is not available, an insecticide-susceptible strain of *Aedes aegypti* may be readily bred and used. Alternatively, 3-day old *Musca domestica* females may be used, but these are less susceptible than mosquitos to dichlorvos vapours.

The cages should be introduced into the room for a standard period of exposure—constant throughout the test series—of 4, 6, or 12 hours (at the same hour on each testing occasion). Mortality should be recorded at the time of removal of the cages from the room tested ; the cages should then be taken to an insecticide-free room, laid on their sides with a piece of cotton damped in sucrose-water on top, and 24-hour mortalities recorded.

It is very important that minimum and maximum temperatures and humidities be recorded for the time when the caged insects are being exposed, as well as the approximate volume of the test room, time at which the test was carried out, and age of the dispensers.

Annex 16

INSTRUCTIONS FOR DETERMINING THE IRRITABILITY OF ADULT MOSQUITOS TO INSECTICIDES

1. Introduction

(*a*) It has been observed that *anopheline and culicine mosquitos* resting on residual deposits of DDT are excited to fly away from them in a comparatively short time, whereas they do not show this response to dieldrin or gamma-BHC.

(*b*) Species will be found to differ naturally in their irritability, some remaining a long time before being excited to fly, while others are irritated into flight almost immediately. It is possible that different populations or strains of the same species will also be found to differ in this characteristic.

(*c*) The purpose of the following test is therefore to measure the irritability to insecticides of a given sample of a mosquito population. It consists of counting the number of take-offs in a given period of time (15 minutes). The test surfaces are the standard impregnated papers devised for the adult-mosquito susceptibility test by WHO; at present only 2% DDT papers are included in this kit, but the method does not preclude the use of other insecticides in the future, with the object of comparing the irritating characteristics of various chemical compounds.

(*d*) Mosquitos used in the test are pre-conditioned for 30 minutes at a standard light intensity. Groups of 5 mosquitos are exposed at a time. With very irritable species, it may prove better to expose them one at a time. For such species, the time to first take-off can be determined.

2. Composition of test kit

The kit includes : 30 small tubes for the pre-exposure of the mosquitos, a special tube-carrying box, three light-proof boxes containing exposure chambers, and three angled aspirators.

(*a*) *Pre-exposure tubes (or adaptation tubes)*. These are small cylinders (5.5×3 cm) made of transparent plastic ; one end is closed by a strip of the same material fitting into a groove ; the other end carries a filter-paper disc soaked in mineral oil ; it constitutes the only internal surface of the tube on which the mosquito settles.

(*b*) *Tube-carrying box* (Fig. 1). This is made of light wood, 45.5 cm long, by 15.5 cm high, by 7.5 cm deep, and opens along two of the long sides. It includes 30 cylindrical compartments; in each of these a pre-exposure tube is placed horizontally; all the bottoms of the tubes (on the side where the paper is placed) are illuminated by a uniform light source of the same intensity as that illuminating the exposure box. The inside of the box is painted black.

By means of the pre-exposure tubes and the tube-carrying box, it is thus possible to reproduce in each test (for a total of 50 mosquitos), the main experimental conditions of the test, i.e., illumination by transmitted light and a contact surface consisting of filter paper.

(*c*) *Light-proof boxes* (Fig. 2). These are made of light wood, 13.5 cm wide by 13.5 cm high by 9.0 cm deep. Light can pass through a circular hole (9 cm in diameter) in the back of the box. Inside there are three grooves, holding: (i) a sheet of translucent glass to ensure uniform illumination; (ii) the filter paper, placed on another sheet of glass; (iii) the exposure chamber, the opening of which is closed by a sheet of transparent plastic. The top of the box is movable and the front consists of two doors; all the internal surfaces are painted black. Two such light-proof boxes with an exposure chamber are used for tests with the insecticide, while a third is used exclusively for observing controls.

(*d*) *Impregnated papers.* Four packages each containing 8 papers impregnated with 2% DDT, and 2 packages impregnated with oil alone for the controls.

3. Experimental technique

(*a*) Introduce one mosquito into each of the pre-exposure tubes by means of an aspirator or by catching the insects directly with the tubes.

(*b*) Place the pre-exposure tubes in the tube-carrying box and illuminate the adaptation surfaces with a source of light as uniform as possible and of the same intensity as that to be used for illuminating the exposure surfaces.[1]

---

[1] For the recommended light intensity of 8 foot-candles to reach the mosquito through the paper, the following distances (in cm) from the apparatus are necessary for naked light-bulbs of three types:

Type of bulb	Distance (cm) from apparatus for bulb of		
	40 watts	60 watts	100 watts
Clear	25	40	50
Frosted	41	55	92
G. E. shadowban	46	66	92

FIG. 1. TUBE-CARRYING BOX WITH PRE-EXPOSURE TUBES

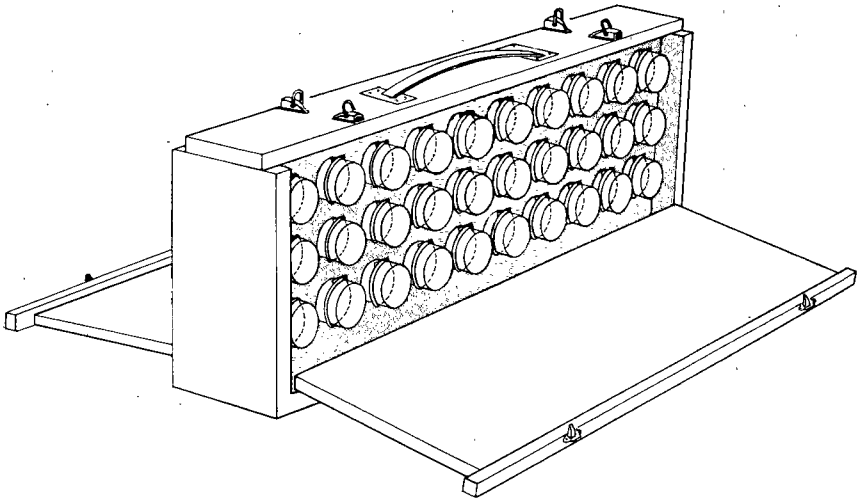
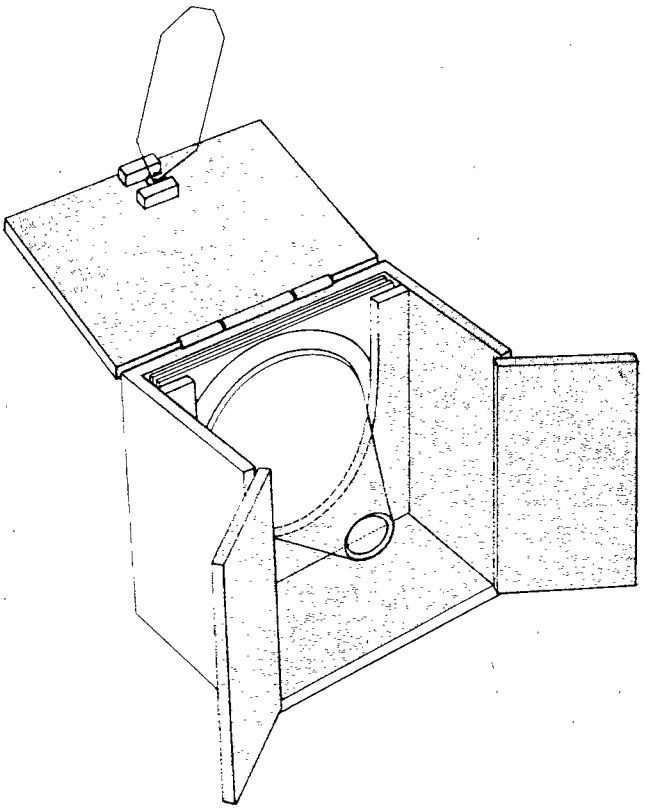


FIG. 2. LIGHT-PROOF BOX WITH EXPOSURE CHAMBER



(*c*) Leave the mosquitos under the conditions described for 30 minutes. After this period has elapsed, all the insects are, as a rule, adapted to the experimental environment. This completes the preliminary treatment and the test can then be commenced (Fig. 3).

(*d*) Transfer 5 mosquitos from the pre-exposure tubes to the exposure chamber ; if the openings in the tube and the chamber are placed in contact, the mosquitos fly almost instantaneously from one to the other (Fig. 4) and on to the filter paper. If any difficulty is encountered in transfer, use an aspirator.

(*e*) Wait for three minutes. During the following 15 minutes count the number of take-offs. In the event of obstinate cone-resting, put the mosquitos concerned back into circulation by the most gentle method that is effective.

(*f*) Each test comprises the exposure of 40 mosquitos (8 lots of 5) to paper impregnated with 2% DDT, and of 10 mosquitos (2 lots of 5) to the control paper.

4. General remarks

(*a*) In any one set of tests, all the mosquitos must be either engorged or else unfed. If they have been collected as adults in the field, they should be brought to uniform nutritional status. Alternatively, they may be laboratory-reared from field-collected larvae ; in this case, the adults should be tested approximately three days after emergence. It is expected that laboratory-reared adults will be found to be less irritable than field-collected adults.

(*b*) The populations whose irritability is being studied should also be tested for their insecticide-susceptibility by the standard test for adult mosquitos.

(*c*) If the irritability of the mosquitos is so great that 5 cannot be counted accurately, they should be tested one at a time, recording the number of take-offs for each mosquito. In this case, it is also possible simultaneously to record the time to first take-off of the single mosquito after the 3-minute settling time ; the actual figure recorded is the time from the initial introduction, i.e., Z + 3 rather than Z. This technique to determine time to take-off of 1 mosquito is also useful to supplement the tests performed on number of take-offs of 5 mosquitos.

(*d*) The records of take-offs from the control paper are also of considerable significance, as they reveal whether or not there are large variations between the populations tested and indicate the magnitude of the difference between the control mosquitos and those exposed to impregnated papers. If the inter-population variations in the controls are large, or if the differ-

FIG. 3. COMPONENTS OF THE IRRITABILITY TEST KIT WITH THE EXPOSURE CHAMBER AND LIGHT SOURCE IN POSITION

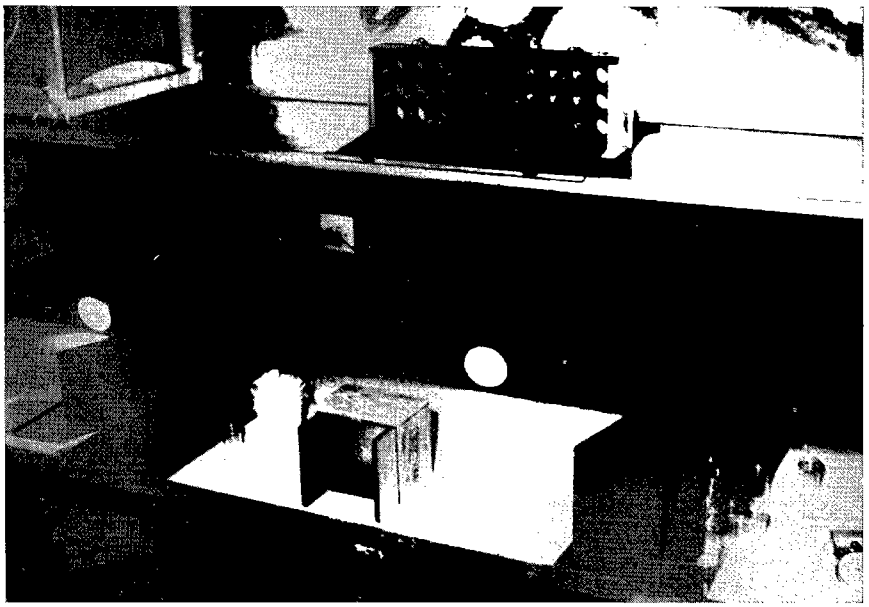
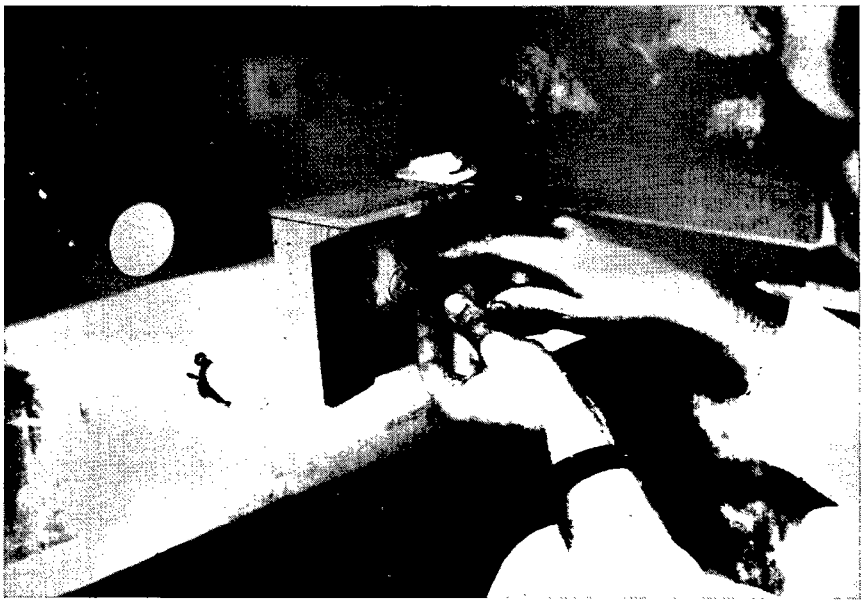


FIG. 4. TRANSFER OF THE MOSQUITO FROM THE PRE-EXPOSURE TUBE TO THE EXPOSURE CHAMBER



ence between the controls and the exposed mosquitos is small, the significance of the test is reduced.

5. Results

(*a*) Record the results on the form provided. Calculate the mean and standard error of the results.

(*b*) The conditions of temperature and humidity obtaining during the exposure period should be recorded.

6. Distribution of reports

One copy of each report form as completed should be sent to the World Health Organization, Vector Control Unit, Geneva, Switzerland. Reports of tests on anopheline species should be addressed to the World Health Organization, Division of Malaria Eradication, Geneva, Switzerland. A second copy of the completed form should be sent to the appropriate WHO Regional Office.[1]

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[1] Addresses of WHO offices are as follows:

Headquarters:

World Health Organization, Geneva, Switzerland

Regional Offices:

World Health Organization, Regional Office for Africa, P. O. Box No. 6, Brazzaville, Republic of the Congo

World Health Organization, Regional Office for the Eastern Mediterranean, P. O. Box 1517, Alexandria, Province of Egypt, United Arab Republic

World Health Organization, Regional Office for South-East Asia, World Health House, Indraprastha Estate, Ring Road, New Delhi, India

World Health Organization, Regional Office for the Americas (Pan American Sanitary Bureau), 1501 New Hampshire Avenue, N. W., Washington 6, D. C., USA

World Health Organization, Regional Office for Europe, 8 Scherfigsvej, Copenhagen Ø, Denmark

World Health Organization, Regional Office for the Western Pacific, P. O. Box 2932, Manila, Philippines

Specimen Report Form

WHO TEST FOR INSECTICIDE-IRRITABILITY IN ADULT MOSQUITOS

Date : .....

1. Investigator : ..... 2. Species : .....
3. Country : ..... 4. Province : .....
5. Condition of mosquito : engorged/unfed [1]
6. History of mosquito : field-collected/laboratory-reared [1]

Individual mosquitos				Groups of 5 mosquitos	
Time lapse before first take-off (minutes)		No. of take-offs (in 15 minutes)		No. of take-offs (in 15 minutes)	
2% DDT	Control	2% DDT	Control	2% DDT	Control
1.	1.	1.	1.	1.	1.
2.	2.	2.	2.		
3.	3.	3.	3.		
4.	4.	4.	4.	2.	2.
5.		5.			
6.		6.		3.	
7.		7.			
8.		8.			
9.		9.			
10.		10.		4.	
11.		11.		5.	
12.		12.			
13.		13.			
14.		14.		6.	
15.		15.			
16.		16.			
17.		17.		7.	
18.		18.			
19.		19.			
20.		20.		8.	
Average					

[1] Cross out what does not apply.

Remarks : ..... Signature of investigator : .....

One copy of this form to be sent on completion to : World Health Organization, Vector Control Unit, Division of Environmental Health, Geneva, Switzerland.

Reports of tests on anopheline species should be addressed to : World Health Organization, Division of Malaria Eradication, Geneva, Switzerland.

A second copy of the completed form should be sent to the appropriate WHO Regional Office.

Annex 17

RECOMMENDED METHODS FOR VECTOR CONTROL

1. GENERAL CONSIDERATIONS

The following is the second revision [1] of a compilation of methods employed to control arthropods and other vectors of medical importance. It has the dual purpose of (*a*) presenting effective methods which can be used in control operations, and (*b*) providing a basis for the interchange of

[1] The original version and the first revision appeared respectively in the eighth and tenth reports of the WHO Expert Committee on Insecticides (*Wld Hlth Org. techn. Rep. Ser.*, 1958, **153**, 15; 1960, **191**, 55)

information among research and operational groups on control procedures and their effectiveness in different geographical areas. The methods below have been obtained through the co-operation of research laboratories, from the literature, from members of the WHO Expert Advisory Panel on Insecticides, and from other sources. Reference is made only to methods that have been proven effective and to pesticides that are commercially available. Emphasis has been placed upon those measures that provide maximum control effectiveness and safety. Pesticides commonly employed in vector control entail varying degrees of hazard to the exposed human and animal populations; the precautions to be observed in their use are given throughout the text along with the recommended procedures, as well as in section 14 (page 206). The common and chemical names of pesticides referred to in this report are listed in section 17 (page 224). Information regarding toxicants not on the commercial market can be obtained from the Vector Control Unit, World Health Organization, Geneva, Switzerland.

It is the understanding of the Committee that these recommendations will be revised periodically, and that in the future periodic releases of information on vector control will be made to keep abreast of changes in these recommendations. In this manner the refinement of old techniques, the development of new pesticides and methods of applying them—such as the residual fumigant technique using DDVP as the toxicant—research on biological control, and the development of new approaches to control, particularly the use of chemosterilants, may enable new recommended methods to be added to those already available.

Despite the phenomenal advances in the development of pesticides, it would be wrong to assume that procedures based solely on pesticides are the principal techniques to be used in operational programmes. In any such programme, environmental sanitation may be the key to successful control. Chemical control alone with no attempt to reduce available larval habitats may yield disappointing results. More permanent measures, such as reduction of mosquito breeding sites by draining, flooding or filling, or the reduction of fly breeding through proper storage and disposal of manure, vegetable refuse and garbage, are basic tenets for accomplishing control. Environmental sanitation procedures are included in this revision under appropriate headings. While pesticidal measures are subordinate to adequate sanitation measures, maximum control cannot usually be achieved without chemical aids, either as a supplementary or an emergency phase. Although this is true for arthropod and rodent control, it does not apply to disease control, which can and has been accomplished in some countries with pesticides alone, e.g., by measures directed at only a part of the insect population, such as mosquitos entering habitations.

In utilizing the measures outlined herein, it is important that the limitations of individual treatments be recognized. Although emphasis is placed on those methods that have proved successful in many different

areas, some control measures may relate to specific geographic areas with certain climatic characteristics and to physiological and behaviouristic qualities of a particular strain of a vector. Thus, measures effective in one country may be of less value in another.

In order to obtain the maximum control with maximum safety and minimum costs, operators should have a knowledge of the species with which they are concerned and a knowledge of the various procedures and pesticides available to them. Application of pesticides on a non-selective basis using routine methods based on a single technique or chemical may lead to unsatisfactory or costly results. For example, the application of insecticidal fogs before dusk is unsuccessful in controlling certain species of mosquitos ; whereas the same treatment at dusk, or shortly thereafter, gives excellent results. Fogging in unfavourable weather conditions can be valueless. Some species of anophelines (e.g., *Anopheles sergenti* in Israel and Jordan) are semi-domestic in their habits, and residual treatments are only partially effective in reducing or interrupting malaria transmission. However, in the same countries a domestic species (*A. sacharovi*) has been almost completely eradicated by similar applications. While a residual application may provide suitable fly control for extended periods, the most efficient emergency method of coping with infiltrations of heavy fly populations would be the use of space-spray treatments.

In addition to the above considerations, the efficacy of a chemical control measure can be markedly influenced by the following: (*a*) the efficiency of the spraying or dusting equipment, (*b*) formulation and application, (*c*) the nature of the surface to which the formulation is applied and sorption of the pesticide in the material to which it is applied, (*d*) stability and potency of the pesticide, and (*e*) the biotic potential of the species. To achieve maximum success in control operations, full recognition must be given to these aspects in the field evaluation of any control programme. In addition, it is emphasized here that all control procedures should be adequately supervised by competent personnel if they are to be successful.

Recommended specifications for spraying and dusting apparatus, established by the WHO Expert Committee on Insecticides for use in malaria control programmes and mosquito larviciding, as well as for pesticides and their formulations, are available from the Distribution and Sales Unit, World Health Organization, Geneva, Switzerland. In the recommended methods given below, information is included on the types of equipment and methods of application to be used. To assist operators in preparing formulations and in determining rates of application, data on these phases are given in section 15 (page 217). It has been shown by some workers that insecticidal efficacy is lost much more rapidly on some surfaces than on others. Detailed studies of this phenomenon have established that the loss of efficacy is often due to sorption of the insecticide below the surface and out of reach of resting insects. Although some light

has been thrown on such aspects as the mobility of the insecticide in the material and the availability of the insecticide at the surface under different climatic conditions, combined laboratory and field investigations are needed to determine the extent and significance of the problem. Although it is impossible in these recommended methods to elucidate the specific individual effect of the surface, sorption, potency or stability of the pesticide on its effectiveness, the indicated duration of the effectiveness of the recommended dosage of insecticide in general takes into account all these factors. The toxicity of a pesticide, its cost, transportation requirements, and the equipment available for its dispersal may all affect the choice of the formulation.

The greatest hindrance to chemical control of certain arthropod species is the occurrence, through chemical selection, of changes in the genetic composition of their populations. These changes may result in increased general tolerance or specific resistance to certain pesticides resulting in failure of control operations in the field. Many species of public health importance, including mosquitos, flies, cockroaches, bedbugs, lice, ticks, fleas and *Culicoides* midges, have been reported as being physiologically resistant in certain parts of the world to one or more of the insecticides commonly employed in their control. Resistance to organophosphorus and carbamate compounds as well as to the chlorinated hydrocarbons has been reported. Fortunately, there are many areas where pest or vector species continue to remain susceptible to insecticides even though, in some instances, these have been in intensive use.

The detection of resistance should be an integral part of control operations, since this is the only way to establish that control failure is, in fact, caused by resistance—and not by other factors—and to confirm the need for a different pesticide.

Detection of resistance can be accomplished without detailed laboratory evaluation, and kits for measuring susceptibility levels of adults and larvae of a number of vectors (mosquitos, body lice, bedbugs, fleas and sandflies) are available from the Vector Control Unit, Division of Environmental Health, World Health Organization, Geneva, Switzerland. Provisional procedures for detection of resistance in tsetse flies, houseflies, blackflies, *Stomoxys*, tabanids, *Triatoma*, and ticks have been selected. Preferably, resistance-detection tests should be initiated before control operations are begun in order to obtain adequate base-line data. Where this is not possible, data on the susceptibility of a species may be obtained by the comparison of mortalities of specimens from treated and untreated areas. As a standard operational procedure, it is desirable that susceptibility levels be measured at periodic intervals. It should be emphasized that the final decision to change insecticides because of resistance should be based not on susceptibility-level determinations alone, but also on field evaluation of the results of control operations.

In control operations, insecticidal measures against both the adult and larval stages will provide maximum initial reduction of the population. However, exposure of both stages may exert a greater selection pressure than exposure of either stage alone and hasten the development of resistance. Control measures affecting both sexes may be more selective than those affecting one sex only. Larvicidal treatment alone may also increase the chances of inducing resistance, since the exposed population is " concentrated " in contrast to the relatively " dispersed " state of adult populations; thus maximum selection pressure is applied. It has been suggested, therefore, that an insecticide employed as a larvicide should be selected from a different group to the adulticide of choice. Fortunately, resistance to chlorinated hydrocarbons is not necessarily associated with resistance to the organophosphorus compounds; however, laboratory studies have shown that selection for resistance to some organophosphorus compounds can also produce high levels of resistance to DDT, dieldrin and other chlorinated hydrocarbons, even though there has been no exposure to the latter compounds. A knowledge of the patterns of cross-resistance is therefore important in the proper selection of insecticides.

Included in these recommendations is a general statement about the status of resistance of each vector or pest species. The recommendations give the insecticide of choice for susceptible species and alternative control measures where resistance has been encountered. With a knowledge of the build-up of resistance in populations of insects and with a choice of alternative treatments, it is possible to draw up a time-table for a succession of insecticides to give control for a predetermined period of time.

2. FLIES

2.1 Houseflies

Houseflies are important filth pests and can usually be found in abundance in most parts of the world. In addition to causing annoyance, they are exceptionally efficient mechanical spreaders of disease, since they breed in, feed on, or walk on materials such as manure, human faeces, garbage and sputum and can transport micro-organisms on their feet or hairy legs, and in their excreta or regurgitated stomach contents. They may carry the micro-organisms causing ophthalmia, dysentery, typhoid, cholera, tuberculosis and yaws—in fact most diseases that can be spread by mechanical contamination.

2.1.1 *Sanitation*

Despite the advances in the development of insecticides, these should not be allowed to take the place of sanitation in the control of houseflies. Since flies breed in moist, decaying organic matter, including animal and human faeces, straw, spilled feed and garbage, and since the development

from eggs to full-grown larvae requires about 4½ to 11 days, sanitary practices can reduce flies by eliminating their breeding places. Spilled animal feed, vegetable refuse and garbage should not be allowed to accumulate in the open; these wastes should be cleaned up promptly and composted, buried, burned or kept in tightly closed containers until they can be disposed of. The proper handling of manure is an important step in fly control. Any process that allows the manure to become dry, such as spreading thinly over fields, or making thin cakes and spreading these out, will prevent fly breeding. Composting, if correctly done, will prevent fly breeding,[1] but improper composting will add to the fly problem. If composting is impractical the manure should be stored in boxes or pits where flies are unable to reach it. Another helpful method is to pile manure in a rectangular stack, preferably on a concrete base. If the manure is packed down and the sides of the stack kept vertical, heat generated within the stack will kill many maggots and drive the rest to the surface. This is an excellent agricultural practice, since it increases the fertilizer value of manure. A ditch dug around the stack and filled with crude oil will catch and kill maggots as they fall. A field experiment has shown that fly breeding may be prevented or controlled by covering the breeding media with a tarpaulin or plastic sheet.

Well-fitting screens on windows and doors—screened doors should swing outward—will give considerable relief from houseflies. Screens with 14 meshes to the inch will keep out houseflies, but finer screens will also keep out some other insects. In humid climates screens of plastic, copper, bronze, aluminium, or one of the rust-resistant alloys should be used; in dry climates galvanized screens are satisfactory.

Electric grids consisting of parallel wires, about 6 mm apart, connected to a high-voltage, low-amperage circuit, help in the elimination of flies by electrocuting those that land or try to pass through. These grids can be mounted in the open or attached to windows and doors. A bait such as molasses, milk or fruit can be used to attract flies to the grid.

Since some fly breeding usually occurs despite the best efforts at sanitation, the use of insecticides is usually required to maintain a high degree of fly control. Insecticides belonging to the chlorinated hydrocarbon, organophosphorus and carbamate groups have been used with success. These insecticides may be applied as residual applications, solid or liquid baits, cords or ribbons, space or contact sprays, and larvicides, although under most conditions larvicidal treatment may not be desirable (see section 2.1.6). Houseflies have been reported resistant to the chlorinated hydrocarbons, many of the organophosphorus insecticides and one of the carbamates. Some countries restrict the kinds of insecticides that may be used

[1] Gotaas, H. B. (1956) *Composting : sanitary disposal and reclamation of organic wastes*, Geneva (*World Health Organization : Monograph Series*, No. 31).

in dairy barns or other situations where they might contaminate milk. In the control of adult flies, consideration must be given to their resting habits, e.g., in some areas a larger proportion of flies may rest outdoors than in other areas.

2.1.2 *Residual treatments*

Residual treatments provide the most effective control of flies where the problem is not complicated by resistance. Sprays used to apply residual treatments may consist of solutions of the insecticide in a solvent such as deodorized kerosene, an emulsion made by adding a concentrated emulsifiable formulation to water, or a suspension of a concentrated wettable powder in water. Around homes, sprays should be applied to garbage cans, screens, porches, doors, window frames, edges of arches, and other places where flies gather. A residual spray may also be used inside the home; but if a house is well screened and a residual spray has been used outside, it is usually easier to kill flies indoors with a space spray. Around farm buildings sprays should be applied to all surfaces where flies rest, including not only ceilings and walls of buildings, but also the surrounding vegetation, fences and garbage cans. Enough spray should be applied to wet the surface to the point of run-off. Although the amount varies with the nature of the surface and to some extent with the formulation, usually 4-8 litres of finished spray per 100 m^2 (1-2 US gal or 0.8-1.6 Imp gal per 1000 ft^2) [1] will be required. Power sprayers, compressed air sprayers or stirrup pumps can be used. Specifications for these can be obtained from the Distribution and Sales Unit, World Health Organization, Geneva, Switzerland.

The insecticides shown in Table 1, when used as emulsions or wettable powders, have proved effective in controlling houseflies.

Where resistance is not encountered, a single application of DDT gives control lasting from several weeks to several months. The residual action of lindane, methoxychlor, toxaphene and chlordane is usually not as long as that of DDT; however, a single application will usually give control of DDT-resistant flies for a few weeks, but since these insecticides are also chlorinated hydrocarbons, the DDT-resistant flies quickly become resistant to them too. In a number of cases (e.g., in antimalaria campaigns), the use of dieldrin as a residual spray has resulted both in a rapid development of resistance to dieldrin in houseflies and in a considerable increase for two to three generations in the biotic potential of the female housefly. The organophosphorus compounds should give

[1] Conversion factors for metric and English units, with the abbreviations used in this annex, are given in section 18. For practical purposes in these recommendations, 1 m^2 is considered equivalent to 10 ft^2; 4 litres to 1 US gal or 0.8 Imp gal; and 1 g per m^2 to 100 mg per ft^2.

TABLE 1. CONCENTRATIONS AND DOSAGES OF INSECTICIDE SPRAYS FOR THE CONTROL OF HOUSEFLIES

Insecticide	Spray concentration	Dosage	
		g per m^2	mg per ft^2
DDT	5%	2	200
Lindane	0.5%	0.2	20
Methoxychlor	5%	2	200
Chlordane	2%	0.8	80
Toxaphene	5%	2	200
Dieldrin	0.625-1.25%	0.25-0.5	25-50
Malathion	1%	0.4	40
Diazinon	1%	0.4	40
Ronnel	1%	0.4	40
Dimethoate	1%	0.4	40
Dibrom	1%	0.4	40

control of flies that are resistant to the chlorinated hydrocarbons, but in some countries resistance has developed to organophosphorus insecticides also. Their residual action is not as prolonged as that of DDT, varying from several days to seven weeks or more. In Denmark, fenthion has been shown to be effective at the rate of 1 g/m^2 in piggeries and in cowsheds (the animals being kept outside during spraying).

In some countries restrictions are placed on the use of certain pesticides ; for example, in the USA the use of DDT is no longer permitted in dairy barns or other situations where it might contaminate milk or food. It can be used, however, in other farm buildings and outdoor situations. Chlordane and toxaphene are not approved for use in dairy barns because of residues in milk. Lindane is approved for use in dairy barns but not in milk rooms. Methoxychlor is approved for use in milk rooms.

None of the organophosphorus insecticides is accepted for complete treatment of the interior of houses. Diazinon and ronnel can be used in dairy barns, including milk rooms, meat-packing and other food-processing plants. Malathion can also be used in dairy barns and meat-packing establishments, but in other food-processing plants and milk rooms it is acceptable only when the premium-grade material is used. Dibrom is labelled for use in dairy barns but not in milk rooms. Dimethoate is acceptable for treatment in dairy barns and poultry houses, but should not be used in milk rooms. The maximum strengths of sprays permitted are : diazinon, Dibrom, dimethoate and ronnel, 1% ; malathion, 5%.

When applying insecticides for fly control, care should be taken that food for humans or animals and animal watering troughs are not contaminated. Milk rooms or food-processing areas should not be treated while in operation.

2.1.3 *Baits*

Poisoned baits control houseflies in some places where sprays fail, including unscreened dairy barns in some areas. If applied properly they can be used in most farm buildings without harming animals or contaminating milk. Baits may be used as scattered dry baits, liquid baits, paint-on baits, or in bait stations. Commercially prepared baits are often available and are usually satisfactory and convenient. If they are not available, they can be prepared from emulsifiable concentrates or wettable powders of malathion, diazinon, ronnel, dichlorvos, trichlorfon, Dibrom, dicapthon or dimetilan.

Dry baits are made to contain 1-2% of the insecticide with sugar. Colouring of the sugar, for example by adding a little lamp-black so that it will be a dirty grey and will not be mistaken for ordinary sugar, is recommended. On moist surfaces, where sugar would dissolve, a cornmeal bait can be used. This is prepared as follows: To one pound of coarsely ground cornmeal, slowly add with stirring one tablespoonful of peanut oil, six tablespoonfuls of a 25% wettable powder or three tablespoonfuls of a 25% emulsifiable concentrate of the insecticide, and two ounces of sugar. Stir with a paddle until all the meal particles are coated with the sugar and insecticide. Five minutes' stirring ensures proper mixing of quantities of one to five pounds; mixing of larger quantities by hand is not recommended. The effectiveness of baits depends to a large extent on the selection of the most suitable formulation and its proper application. Baits should be applied liberally in areas where flies feed and congregate. The sweetening agents in the baits are not attractants in the true meaning of the word, but rather they are arrestants. Consequently it is necessary to have the baits so well scattered that the flies can hardly fail to find them. Baits should be scattered finely, for flies will feed only at the edges of a clump of bait, much of the bait thus remaining ineffective. The poisons act quickly and spectacular immediate kills are often seen. It is usually necessary to re-apply the baits daily for several weeks until fly breeding in the area has been greatly reduced. Thereafter it may be sufficient to make applications twice a week, but if fly infestations increase, daily application should be resumed. Dry sugar bait is applied from a shaker-top can or glass jar and sprinkled in narrow strips on floors, walkways, window sills and other places where flies gather, usually about 60 g per 100 m^2 (2 oz per 1000 ft^2). It should be applied only on dry, firm surfaces. Cornmeal bait is applied about twice as heavily as the sugar bait and used on slightly moist surfaces. If flies are numerous, heavier applications may

be necessary. If satisfactory control is to be obtained with baits, it is necessary to observe the locations in which the flies are congregating and feeding and to apply the baits in these locations by the method most suited to the character of the ground, floors or walls.

Liquid baits are made with water containing 10% sugar, molasses, or sirup and 0.1-0.2% insecticide. Application can be made with an ordinary sprinkling can with about half the holes plugged so that the bait will be spread thinly in strips 4-6 inches wide. Liquid baits are particularly suitable for application to concrete floors ; for other surfaces, covered with dirt or litter, the bait should be sprinkled on sheets of tin, wood, paper or other material. The application rate is about 4 litres per 100 m^2 (1 US gal or 0.8 Imp gal per 1000 ft^2), but if infestations are very heavy larger amounts may be needed.

Neither a dry nor a liquid bait gives good control of flies in animal pens where the ground is muddy, trampled or littered, but a paint-on bait will usually give good control in these places. It is applied with a paint brush to suitable fly resting places such as posts, railings or fences. Fences around pens should be treated only on the outside. A 1-2% mixture of the insecticide with molasses, corn sirup or a thick sugar-water slurry makes a satisfactory paint-on bait which will remain effective for a week or more unless destroyed by rain. Paint-on baits may also be used in barns where they are applied to window frames or other spots where flies like to rest. Another method is to apply the bait to a burlap sack, clip it into pieces and suspend them in the barn or pen. A sugar solution may also be used and applied with an ordinary knapsack sprayer.

Bait stations are useful for the control of flies gathering on manure or muddy surfaces or for the treatment of barns or accumulations of manure under caged poultry. Two types of bait stations have been developed—one dry, the other liquid. The dry bait stations, as well as the dry and liquid baits mentioned above, have certain advantages over liquid bait stations. A large number of liquid bait stations are required to cover a given area, they contain a reservoir of insecticide relatively attractive to chickens, dogs, etc., and they are liable to be knocked over and spilled. Dry bait stations, however, are versatile and they can be fixed almost anywhere by stapling or by pushing the handle into a soft surface, so that they can be kept away from small children and animals.

A convenient dry bait station consists of a paddle made by fastening a four-inch square of screen wire to a slender wooden handle about six inches long. The wire is coated with a bait consisting of 50% of sugar, 46% of sand, 2% of the insecticide and 2% of gelatin or, better still, bacto agar. To prepare the bait the sugar is first mixed thoroughly with sand. Boiling water is poured slowly over gelatin or agar with stirring until it has absorbed as much as it will hold and starts to liquefy. The liquid is poured over the sand-sugar mixture and stirred thoroughly. The insecticide is then added

and thoroughly mixed in. If necessary, additional water may be added to make a putty-like mixture which can be spread on the screen wire without running off. Paddles should be allowed to dry for about 24 hours.

In use, the wooden handles of the bait stations are thrust into the soil around the edges of pens or into the manure in poultry houses, so that the baits are held in a vertical position with the lower edges just touching the surfaces. They should be spaced 5-10 feet apart where flies are numerous. They will remain effective for several weeks unless destroyed by rain. The bait stations can also be used inside dairy barns, where they can be tacked along walls or on window frames. They are especially useful in feed rooms, where they can be laid on sacked feed without danger of contamination. About 50 well-placed stations will provide control in most barns. They take longer than scattered baits to produce a high degree of control, but a single application remains effective for several weeks.

An effective device for dispensing liquid baits is a chicken-watering unit modified by inserting a cellulose sponge in the trough to prevent its becoming clogged by dead flies. Toxicants such as dichlorvos or trichlorfon at a concentration of 0.1% in sugar-water solution have been effective. In recent tests, a solid formulation of dichlorvos in a base of 25% dibutyl phthalate and 75% Montan wax No. 16 affixed in the melted form to the bottom of the reservoir jar was effective in controlling houseflies. From June to October the sugar-water (12.5%) was replenished five times; the dichlorvos was not replenished during this time. A long-established method still occasionally used is the exposure in saucers or other shallow containers of a mixture of sugar and milk containing 1% formalin as a poison bait for houseflies.

In some countries baited fly traps with or without a poison bait have met with a degree of success as a method of fly control.

2.1.4 *Cords and ribbons*

In some regions the use of cotton cords or strips treated with diazinon or parathion has given season-long control of flies. In other regions, perhaps because of a difference in the behaviour of the flies, the control achieved in this manner has not been satisfactory. Cords and strips impregnated with diazinon and parathion are available commercially. Because of the toxicity of the chemicals, it is recommended that cords be prepared by experienced personnel only. The directions for use as printed on commercially available packages should be carefully followed. Gloves should be worn while working with cords or ribbons, which should be hung like festoons from ceilings, allowing about 1 m for each m^2 (30 linear ft per 100 ft^2) of floor area. Other insecticides, such as ronnel, fenthion, dimetilan and diazinon have been satisfactory in these types of treatment.

2.1.5 *Space sprays*

Space sprays are finely-divided mists or aerosols and can be applied indoors or outdoors using hand or power sprayers : outside they may also be dispensed by portable, thermal aerosol generators (e.g., the " Swingfog " apparatus) or sprayed from aircraft. These sprays in general have little or no residual action and produce only a temporary effect on adult fly populations ; consequently, repeated applications are necessary. Since the adult kill depends on contact with the insecticidal droplets, treatment must take into account the activity pattern of flies and the site to be treated.

Flies that are susceptible to the chlorinated hydrocarbon insecticides can be killed with emulsions or fuel oil solutions of DDT (5.0%), chlordane (2.5%) or lindane (2%). If avoidance of odour is not important, a 5% technical BHC emulsion (12% of the gamma isomer) is cheaper than lindane and equally effective. Chlordane, dieldrin and lindane are also effective against DDT-resistant flies, but flies may become resistant to these also.

Organophosphorus compounds are effective against houseflies resistant to the chlorinated hydrocarbon insecticides, but the dosages required for satisfactory kills are relatively high. Limited tests with malathion indicate that dosages of 560 g per ha (½ lb per acre) or higher are required. The effective swath width in these tests was less than 90 m (300 ft), consistently high kills being obtained with 30 m (100 ft).[1] Aerial sprays of Dibrom emulsions containing 10% sugar applied at a dosage of 112-224 g per ha (0.1-0.2 lb per acre) in Florida produced a 90-97% reduction of houseflies in 24 hours.

Quick knockdown sprays have been used to some extent by dairy- and beef-cattle farmers. Deodorized kerosene solutions of pyrethrum (0.1-0.2% plus 1-2% of a synergist such as piperonyl butoxide, n-propyl isome, or sesame), diazinon (0.1%), ronnel (2%), or malathion (2%), applied with hand or power sprayer or aerosol dispenser produced a 70-98% reduction within 10 minutes. With most of these sprays not more than a 50% reduction was evident 24 hours after treatment, even when the application was repeated daily for 5-21 days.

Other organophosphorus compounds that have been used successfully as space sprays are dimethoate, dichlorvos, diazinon, and Dibrom.

2.1.6 *Larvicides*

Larvicides are of value in fly control for the treatment of garbage or animal excrement that accumulates in privies, stockyards, chicken farms

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[1] Per hectare dosage based on a 30-m (100-ft) swath would be 1680 g (1.5 lb).

TABLE 2. SUMMARY OF RECOMMENDED INSECTICIDES FOR THE CONTROL OF HOUSEFLIES

Insecticide	Formulation [a]	Application
	Residual sprays	
DDT Lindane Methoxychlor Chlordane Toxaphene Dieldrin Malathion Diazinon Ronnel Dimethoate Dibrom	5% EC or WP 0.5% EC or WP 5% EC or WP 2% EC or WP 5% EC or WP 0.625-1.25% EC or WP 1% EC or WP 1% EC or WP 1% EC or WP 1% EC 1% EC	Apply 4-8 litres (1-2 US gal, 0.8-1.6 Imp gal) per 100 m² (1000 ft²) or more with organophosphorus insecticides. See instructions for formulation, section 15; precautionary measures for pesticides, section 14; and restrictions for use as residual treatments, section 2.1.2. Avoid contamination of human and animal food and watering troughs.
	Baits	
Malathion Diazinon Ronnel Dichlorvos Trichlorfon Dibrom Dicapthon Dimetilan	1-2% dry baits on sugar or cornmeal. 0.1-0.2% liquid baits in sugar water.	Apply 57-114 g (2-4 oz) of dry bait per 100 m² (1000 ft²). Apply 4 litres (1 US gal or 0.8 Imp gal) or more of liquid bait per 100 m² (1000 ft²). See text for types and preparation of baits and the use of bait stations for prolonged efficacy. Do not use inside homes.
	Impregnated cords and strips	
Parathion Diazinon	To be prepared only by experienced formulators.	Install at the rate of about 1 m for each square metre (30 ft per 100 ft²) of floor space.
	Larvicides	
Chlordane Lindane Dieldrin Aldrin	2% EC or WP 0.5% EC or WP 0.625-1.25% EC or WP 1-2% EC or WP	Apply 4-8 litres (1-2 US gal, 0.8-1.6 Imp gal) per 100 m² (1000 ft²).
Diazinon Malathion Ronnel Dichlorvos	Use as emulsion concentrates, dusts or kerosene solutions.	Apply 85-170 g (3-6 oz) of actual insecticide per 100 m² (1000 ft²).
Paradichlorobenzene	Technical	Apply 60 g (2 oz) per garbage can.

[a] EC = emulsifiable concentrate; WP = wettable powder

or other problem sites. Larvicides may cause or accelerate the development of resistance. Sanitation rather than larvicides should be considered the first line of defence. In one instance in the USA the treatment of privies with dieldrin destroyed soldier flies, *Hermetia illucens*, which were making privy contents unsuitable for housefly breeding, and the number of resistant houseflies increased rather than decreased. It must be remembered, however, that in hot climates *Musca domestica vicina* breeds intensively in privies. During the hot seasons when other substrates dry out, the privies are the main breeding places for this subspecies. Other species of flies that represent a danger as vectors of intestinal infections also breed in privies. Consequently, the treatment of privies with larvicides may at times be unavoidable.

In using chemical measures against fly larvae, the principal difficulty lies in applying the toxicant in such a manner as to ensure adequate contact between it and the insects. Although flies breed chiefly in the upper layers of a substrate, adequate penetration of media such as excrement or garbage is difficult to achieve. In treating breeding material, it may therefore be advisable to employ a greater volume of a dilute formulation rather than a smaller volume of a concentrated formulation.

Against susceptible housefly strains, chlordane, lindane, and dieldrin serve as effective larvicides. They should be applied as sprays at dosages recommended for residual treatments. Aldrin is also an effective larvicide applied as a 1-2% spray. Of the numerous organophosphorus compounds toxic to housefly larvae, diazinon appears to be the most effective as regards both immediate kill and persistency. Dosage rates of 0.5-1 g per square metre (50-100 mg per m^2) are needed, the formulation being applied at the rate of 28-56 litres per 100 m^2 (7-14 US gal or 5.6-11.2 Imp gal per 1000 ft^2). Successful applications have been made with diazinon in granular carriers such as sand vermiculite. Other toxicants, such as malathion, ronnel, dichlorvos and parathion, are effective against fly larvae. Thiourea at a dosage of 40 g per m^2 has also been used to control insecticide-resistant housefly larvae.

As a larvicide for use by the individual householder, paradichlorobenzene applied at the rate of 60 g or 2 oz per garbage can has been shown to give satisfactory control of fly breeding for periods of 1-2 weeks.

2.2 Tsetse flies

Suggestions for control of tsetse flies by chemical means must show some advantage, in cost or speed of action, over methods of control by total or partial clearance of the bush habitat of the flies. Whenever insecticides are used—except, for example, to secure a short-term reduction in numbers to stop a sleeping sickness epidemic—isolation of the tsetse habitat is essential for permanent results.

2.2.1 *Residual treatment of vegetation*

In East Africa the riverine tsetse *Glossina palpalis* is apparently dependent on vegetation at the water's edge for its feeding area. Treatment of this vegetation with an emulsion of a residual insecticide so that it remains lethal to the fly on short contact for more than a pupal period (say for two months) is generally sufficient to control this species and possibly to eradicate it. The earlier control measures of this nature were carried out with 5% DDT emulsion applied at about 42 litres per km (18 US gal or 15 Imp gal per mile of river bank) four times at intervals of two weeks at a cost of £42 per mile. It was found later that a 1.5% dieldrin emulsion applied twice with a month's interval, or a 5% dieldrin emulsion applied once—in either case at a rate similar to that for DDT—was more effective. In Uganda, the standard method is now to apply 5% dieldrin emulsion once only to selected portions of the riverine vegetation at a total cost of £20 per mile.

These costs compare with an average cost of bush clearing on these rivers of £200 to £300 per mile, but they may be more than doubled by the cost of the necessary surveillance to determine whether any fly remains. In most of this control work, the insecticide has been applied with knapsack or compression sprayers by labourers walking down the river bed, but in some larger streams a more effective method has been found to be application by a power sprayer from a punt which is poled down the river.

The control of the riverine species *G. tachinoides* in Northern Nigeria is a similar but rather more complicated problem. More extensive spraying is required but, because there is a long, completely dry season, DDT wettable powder can be used instead of dieldrin emulsion and this results in a great saving in the cost of the insecticide. The cost per mile of river is about £15.

A considerable amount is now known about the resting places of the savannah species of tsetse fly *G. morsitans* and *G. swynnertoni* in East Africa. In a 15-square-mile block of isolated *G. morsitans* habitat in Uganda, dieldrin emulsion was applied experimentally to the lower side of the branches of *Acacia gerrardii* trees—the putative daytime resting places of the flies—in fly concentration areas; this resulted in a reduction of over 99% in the apparent density of *G. morsitans*. This method, if applied over a sufficiently large area, would probably be effective in eradicating the fly at a cost of about £250 per square mile.

In areas with a very severe dry season, *G. morsitans* becomes essentially a riverine species. For example, in the north of the Northern Region of Nigeria, where the relative humidity is as low as 5% for most of the day during several months of the year and the maximum temperatures are well over 38°C this fly is confined during the heat of the day to the lowest part of the trunks of the well-shaded trees along the river banks. Treatment of

those places with residual insecticide, a method which is comparatively cheap and rapid, is sufficient to eradicate *G. morsitans*. In places where the dry season is neither so long nor so severe, more extensive treatment is required, but the method is still practicable.

2.2.2 *Application by aircraft*

Application from the air is appropriately used against widespread tsetse species, such as *G. morsitans*, *G. swynnertoni* and *G. pallidipes*, but it has been found to be uneconomical—where it is at all possible—against species living in the high forest, such as some *G. palpalis* and *G. brevipalpis*.

Savannah woodland can be treated successfully, even if it contains thickets, although it is preferable to choose the leafless period of the year.

The only effective applications have consisted of the insecticide in the form of a coarse aerosol containing as much as possible of its volume in droplets between 5 μ and 50 μ in diameter. Larger droplets are unable to by-pass obstacles such as leaves and branches and penetrate to the resting sites of the tsetse flies, while smaller droplets do not settle on the insects.

Under tropical conditions, applications of coarse aerosols are effective only for a limited period just after dawn, and are occasionally effective for an even shorter period before dusk. At other times, the meteorological conditions are so unstable that rapid diffusion of the aerosol occurs.

The aerosols have been produced either by high pressure through fine nozzles on an extended boom or by injection of the insecticide solution into the exhaust of the aircraft. The former is the more flexible arrangement and avoids decomposition of the insecticide by heat. Injection into the exhaust system causes some decomposition, but the use of a suitable solvent and care in selecting the point of injection can reduce this to a minimum and droplets of the desired size can be produced.

A typical effective procedure has been to use a 10% solution of DDT in oil applied at 2.8 litres per ha (one Imperial quart or $^5/_6$ US quart per acre) with a swath width of 50 m (55 yd). In East Africa, applications were made at fortnightly intervals for three months or more and, when carried out by Avro Anson aircraft, eight applications cost about £1000 ($2800) per sq mile treated (about £390 or $1080 per km^2). The use of smaller aircraft and more concentrated solutions of insecticide could reduce costs considerably, but as yet reliable results have not been regularly achieved.

In South Africa, eradication of *Glossina pallidipes* was accomplished in Zululand chiefly through aerial application of aerosols of lindane (33.6-112 g per ha or 0.03-0.1 lb per acre) and of DDT (448 g per ha or 0.4 lb per acre).

The total cost of treatment with single-engine aircraft dispersing the insecticide (4% lindane) at the rate of 5.2 litres (1 Imp gal or 1.3 US gal) per minute over an area of 6.1 ha (15 acres) worked out at 3/3 per ha ($0.18 per acre) per application. It was necessary to give 8-10 treatments per year at approximately monthly intervals, a single aircraft covering about 5500 ha (13 500 acres) per month.

2.2.3 *Ground aerosol machines*

Ground aerosol machines could compete directly with aircraft applications for the destruction of savannah tsetse species, but they need a more elaborate organization to cover an extensive area. The "TIFA" fog generator has been used with good results in Northern Rhodesia to apply a thermal aerosol containing 1.5% of lindane to eradicate *G. morsitans* from isolated pockets, the cost of the insecticide being about £100 per square mile. The "Swingfog" generator has been tried in Rhodesia to prevent carriage of tsetse flies by road traffic and in Kenya the "TIFA" has been used to disinfest trains in a similar way.

2.3 Blackflies

Blood-sucking flies of the family *Simuliidae* are vectors of onchocerciasis in Africa, Mexico and Central and South America. In other areas where this disease is not prevalent they constitute a serious pest problem.

2.3.1 *Insecticides*

DDT applied to water-courses where the larvae breed is the method of choice for control of black flies and the reduction or eradication of onchocerciasis. In Kenya the species, *Simulium neavei*, has been eradicated in this manner. Eradication, rather than just control, was possible because the foci were isolated, they were all located, and the programme was well planned and meticulously executed.

DDT applied by the drip method, or by spray applications, as an emulsion, a suspension or an oil solution, will control larval infestations. Although certain cyclodienes (such as heptachlor) and lindane are effective at lower doses than DDT and lindane can kill new pupae, and although parathion may be more effective in slow-moving water, those who have investigated these insecticides in Canada and Guatemala agree that DDT is the insecticide of choice.

The type of formulation, means of application and rate of application depend, to some extent, on the water-course to be treated and its flow and other characteristics. The most suitable formulation is an emulsifiable concentrate because it can carry the most DDT (up to 25%) in liquid form. Solutions of DDT in fuel oil, gasoline or kerosene may spread more rapidly

across a river, but without auxiliary solvent they can carry no more than 5% DDT (with diesel oil perhaps 10-12.5% DDT). The specific gravity of the formulation used has been 0.84-0.98 for DDT in oily solution and 1.00 or slightly above for emulsion concentrates. Emulsifying agents have been added in some cases. With formulations that do not spread as rapidly as light oils and when treating wide water-courses, care must be exercised to ensure that the concentrate is mixed with the water for the entire width of the river. Other schemes for applying DDT to water-courses have been developed. These include plaster blocks, bags of wood shavings or vermiculite impregnated with DDT in oil, gelatine capsules, and pastes. Although these methods may be satisfactory in some situations, they are generally inferior to the use of liquid formulations.

Experience has shown that complicated equipment is unnecessary for application. Any metal container in which a hole or holes can be punched can be made to give the proper delivery rate. In many parts of Africa, four-gallon kerosene cans are readily available. However, when a control scheme requires that applications always be made at the same place and at regular intervals, a commercial constant-flow dispenser may be permanently installed.

The applications need to be repeated at weekly to fortnightly intervals because of the large number of generations that occur per year, the length of the larval stages, and the life span of the adults. At present there is no definite evidence that, in the field, *S. damnosum* or *S. neavei* lives longer than three weeks, except for rare individuals or in regions where estivation may take place. The general experience (e.g., on the Victoria Nile) is that adult blackflies disappear after the third application. In control schemes, where economy in costs of insecticide and labour time are all-important, it is possible that a series of 3 applications at 6-weekly intervals, or triple applications at 6-monthly intervals, may give better control over the year than a single series of 12 applications in one season only. The most important requirements for a control or eradication programme are reconnaissance and accurate recording of results. These permit evaluation of the programme and readjustment of control measures to secure maximum effectiveness.

Rates of application of DDT are given as the amount of DDT required to produce a certain concentration in p.p.m. in water over a given application time. The approximate volume of the stream flow per second is estimated from measurements of the average velocity of the water and the cross-sectional area of the stream at the point of application. The dosage rate for DDT that is both effective and safe for fish may be taken as 0.5 p.p.m. per 30 minutes. A rate of 0.1 p.p.m. per 30 minutes is reliable for large rivers, and probably for most smaller ones. Only in very small streams must the dosage be increased to 1 p.p.m. or higher to allow for loss along the margins.

In most cases any control failure will be found to be caused not by insufficient DDT concentration or too short a period of application time, but to non-carry in slack reaches, to diversion of the treated water on one side of islands, or to obstructions. The number of points of application on a water-course (also the distance the treatment will be effective) should be determined by adequate surveys, since it will vary from area to area and river to river. DDT treatment has given complete or almost complete control over relatively short distances and also up to 160 km (100 miles) downstream.

Application of DDT for larval control has been made by airplane; dosages are based either on a swath area (112-224 g per ha or 0.1-0.2 lb per acre for a swath width of 100 ft) or on the maintenance of a given concentration in water for a certain time (0.13 p.p.m. for 36 minutes).

Continued use of DDT larvicides may produce DDT residues in the visceral fat of fish; it is therefore important that the organophosphorus compounds fenthion, Chlorthion and trichlorfon are safe to fish at larvicidal concentrations and do not leave residues.

Where circumstances warrant chemical treatment against adult blackflies, ground or aeroplane application of 5-10% DDT solutions in fuel oil at the rate of 450 g of toxicant per hectare (0.4 lb per acre) has given satisfactory but temporary control.

2.3.2 *Repellents*

The standard mosquito repellents will prevent blackflies from biting on exposed skin for various periods of time. The protection period will vary with the repellent, the individual, and the amount of sweating, rubbing, or exposure to water. The most effective materials recommended for application to the skin are diethyltoluamide, chlorodiethylbenzamide, dimethyl phthalate, dimethyl carbate, and ethylhexanediol.

Blackflies exhibit a preference for getting inside the clothing and biting there, so that repellent applied to the exposed skin does not always give complete protection. The use of clothing that will prevent the flies from crawling up the sleeves and trouser legs, or into shirt fronts, is advisable. Clothing in light colours, particularly khaki or olive-drab, is less attractive than that in dark colours, such as reds and blues. Additional protection may be obtained by treating the clothing with a repellent as described in section 6.2 for chigger repellents.

2.4 Sandflies and midges

At present there are no substantiated instances of insecticide resistance in phlebotomines. The use of the test method that has been standardized by WHO (Annex 7) has revealed normal susceptibility in all populations of sandflies investigated.

2.4.1 Phlebotomus *(sandflies)*

Adult *Phlebotomus* are eliminated readily from dwellings by DDT anti-malarial sprays at 1 or 2 g per m^2 (100 or 200 mg per ft^2). It is important, however, that these deposits extend down to the floor level. If spraying is carried out specifically against *Phlebotomus*, it may be confined to the entrance hall and bedroom, and exclude the kitchen. Additional spraying around outside entrances and windows is profitable. Also animal shelters, stone walls and other outside resting places may be treated.

Results from Italy, Yugoslavia, Greece, Romania, the USSR, Lebanon, Egypt, India and Peru indicate that a single application remains highly effective for a period of 1 to 2 years. One application of 75% DDT water-dispersible powder to houses and other structures in Bobo Dioulasso, Haute Volta, eliminated *P. dubosqi* between 1955 and 1959. Residual deposits of 0.22 g per m^2 (22 mg per ft^2) of lindane obtained by the spraying of suspensions in homes have substantially reduced *P. antennatus* populations for a period of three months in India.

2.4.2 Culicoides *(midges)*

In North America and Australia the name " sandfly " has been applied to species of the genera *Culicoides*, *Leptoconops*, and *Styloconops* (family Ceratopogonidae). Many of these species breed in moist soil and some are found in fresh-water locations, in tree-holes, and along the banks of ponds and spring-fed streams. The *Culicoides* vector of *Acanthocheilonema* in tropical Africa breeds in rotting banana stumps. Fresh-water species are commonly called punkies, no-see-ums, or biting midges. A few species are found associated with salt-marshes.

The larvae of salt-marsh sandflies are found in very wet organic mire (mud) or sandy soil, usually on portions of the marsh affected by daily tides. Experimental work has shown that chlordane at 2240 g per ha (2 lb per acre) and dieldrin, heptachlor and lindane at 112-1120 g per ha (0.1-1 lb per acre) are effective for control. DDT at 1120 g per ha (1 lb per acre) has been effective for some species. In Florida, USA, after three treatments with dieldrin over three years, the saltmarsh sandfly *Culicoides furens* became resistant to this compound and to related cyclodiene insecticides. The higher dosages of these insecticides may be harmful to fish and wildlife and care should be observed in their use.

Adult *Culicoides* have also been controlled by the use of insecticidal fogs. Screens painted with a 5% DDT or 8% malathion solution will reduce the entry of these insects into houses.

2.5 Tabanids

The large and cosmopolitan family of Tabanidae includes the blood-sucking flies commonly and variously called horse-flies, deer-flies, green-

heads, breeze-flies and mango-flies. These flies can cause severe annoyance to animals and humans. Two species of mango-flies, *Chrysops silacea* and *C. dimidiata*, are proven vectors of *Loa Loa* worms in various West African countries. Tabanid flies may also be mechanical vectors of certain important diseases of animals, e. g., anthrax, tularaemia, and surra. This ability to act as mechanical vectors is connected with the habit of feeding on a succession of hosts to secure a single blood-meal. If disturbed, the flies can proceed to a second host, the wet proboscis carrying disease organisms. It has been shown in certain instances that *Chrysops* can remain infective for a period of days.

Control of tabanids has been and continues to be difficult. The use of the chlorinated hydrocarbon insecticides offers possibilities for control. In Canada, lindane has been applied by aeroplane at the rate of 560 g per ha (0.5 lb per acre) for the control of adult flies on a sandy plateau containing spruce and muskeg. It gave effective, though temporary, control in open forest, but a smaller reduction (82%) in dense spruce forest. Granulated formulations of dieldrin applied at the rate of 336 g per ha (0.3 lb per acre) gave control of tabanid larvae in coastal saltmarsh breeding areas in New York State, and a large-scale application also reduced adult populations. Pyrethrum plus a synergist may be applied to animals as a spray. For the protection of human beings, diethyltoluamide is an efficient repellent.

3. MOSQUITOS

The mosquitos (family Culicidae) constitute the most important single family of insects from the standpoint of human health. Their distribution is world-wide and they have always been known as intolerable pests. However, more important than their pestiferous nature is their role in the transmission of human disease, since they act as vectors of malaria, filariasis, dengue, yellow fever and several of the encephalitides.

Resistance to the chlorinated hydrocarbon insecticides has occurred in many areas and in many species, both anophelines and culicines. Resistance to the organophosphorus insecticides malathion and parathion has occurred in the USA in *Culex tarsalis* and *Aedes nigromaculis*, and in the Cameroons, West Africa, in *Culex pipiens fatigans*. Fortunately, organophosphorus-resistant populations of these species are scarce, and a population showing resistance to one organophosphorus insecticide is not necessarily resistant to others. For example parathion-methyl can be used to control parathion-resistant populations. Despite the increase in resistant species and in the extent of areas where resistant populations have developed, there are examples of long-term use of insecticides without the appearance of resistance.

3.1 Anophelines

3.1.1 *Residual treatments*

Control of adult anopheline mosquitos by the application of sprays of residual insecticides to the interior surfaces of dwellings is the principal measure employed in antimalaria programmes. These sprays are applied to the walls, ceilings and other surfaces to a point just short of run-off; in some cases, spraying of outside eaves may be useful. A rate of application of 4 litres of finished spray per 100 m^2 (approximately 1 US gal or 0.8 Imp gal per 1000 ft^2) is equivalent to spraying to the point of run-off in most cases. Where surfaces are porous, a considerable amount of insecticide penetrates the surface, so that an increase in the amount applied per unit area with a lower strength formulation may be necessary. Table 10, section 15, page 218 gives the relation of spray concentration, rate of application and dosage in g per m^2 (mg per ft^2).[1] Use of this table will allow conversion of spray concentration into applied dosage and will facilitate changes in formulations or application rates necessary for a given applied dosage. Actual run-off should be avoided in the treatment of dwellings. The best formulation, for most conditions, is a water-dispersible powder; besides being easier to transport, these powders are known to be more toxic to mosquitos than other formulations when the toxicant is DDT or malathion. Water-dispersible powders with the highest concentration of the toxicant compatible with other requirements are preferred. Occasionally, where houses are of a high standard, emulsion concentrates or oily solutions are substituted. However, the formulation should not stain or damage the surfaces. Directions for preparation of sprays from wettable powders and emulsion concentrates are given in Tables 11 and 12, section 15, pages 218 and 219.

Of the three chlorinated hydrocarbon insecticides employed in antimalaria campaigns, dieldrin and lindane are more toxic than DDT, and lindane is more volatile than DDT or dieldrin. These properties decide respectively the dosage and the duration of effectiveness of these compounds.

Considerations of toxicity and cost may also influence the choice of insecticide. DDT is most widely used, since it is cheap, almost universally available, has a low toxicity, and is effective under a wide range of conditions. Lindane is highly effective for short periods and its fumigant action is of considerable value. Dieldrin has frequently been chosen in situations where the inaccessibility of the region puts a high premium on extended residual action. However, many anophelines have become resistant to dieldrin within one or two years of its introduction. This dieldrin-resistance

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[1] See footnote, page 161.

extends to lindane but not to DDT. Conversely, in the instances where the vector has developed resistance to DDT, dieldrin has been effective as a substitute insecticide. DDT is irritant to many anophelines and certain populations have developed increased irritability to this insecticide. The importance of this phenomenon in malaria eradication programmes is under continuing investigation.

Since the development of resistance is always a possibility, it is essential that the susceptibility levels of the vectors concerned be determined by the standard WHO test methods during the pre-operational or preparatory phase and reassessed continually throughout the campaign. The results of these tests will indicate whether the population remains susceptible or whether there is a progressive decrease in its susceptibility to the insecticide in use. These results, together with other entomological and epidemiological data, will serve as a basis for deciding whether or not a change in the insecticide is warranted.

In instances where the level of resistance to DDT is such as to exclude its further use, dieldrin or lindane is a satisfactory substitute. In cases of resistance to dieldrin and lindane, DDT is the effective substitute. In an area where there has been a wide use of insecticides, resistance to DDT as well as to dieldrin and lindane may occur. In such situations, a change to malathion can be made; however, this insecticide should be substituted only if DDT, lindane and dieldrin have been conclusively proven to be ineffective as residual treatments.

The dosages and spray cycles for the recommended insecticides vary from country to country; however, those most commonly employed are as follows:

TABLE 3. RECOMMENDED DOSAGES AND SPRAY CYCLES FOR ANOPHELINE CONTROL

Toxicant	Dosage in g/m² (mg/ft²)	Cycle (months)	Duration of effectiveness (months)
DDT	1 or 2 (100 or 200)	6	6-12
Dieldrin	0.5-0.6 (50-60)	12	6-12
Lindane	0.5 (50)	3	3
Malathion	2 (200)	3	3

The selection of the insecticide, along with its dosage and spray cycle, is dependent upon numerous factors which vary with the vector and the geographic area concerned. These factors include:

(*a*) *Efficacy of deposit against the vector.* The duration of effectiveness of the several insecticides should be determined by suitable methods of biological assessment. These include regular measurement of mosquito

densities in sprayed and unsprayed dwellings and the bio-assay of insecticide deposits on treated surfaces by the wall-cage test.[1]

(*b*) *Length of malaria transmission period.* Where the transmission period is limited (less than three months), a single annual application of BHC may be sufficient in areas in which the spray coverage can be completed in a short time; alternatively, lower dosages of the more persistent insecticides may be used, for example, a single spraying of DDT at the rate of 1 g per m^2.

(*c*) *Type of wall and ceiling treated.* Experiments have shown that there may be a comparatively rapid loss of insecticidal action on certain surfaces, such as lateritic muds of high colloid content. However, it seems at present that sorption is not of major importance in any programme, although the evidence is not yet complete. Insecticidal activity is more persistent on surfaces such as thatch, wood or paper. Alkaline surfaces, such as lime and cement, do not destroy the chlorinated hydrocarbon insecticides, but they hydrolyse certain organophosphorus compounds.

(*d*) *Loss of surface deposit.* Removal of deposits by washing or rubbing, or their obliteration by replastering, papering, liming, smoke or soot, may result in losses of active surfaces as high as 25% in six months. In addition, new construction and temporary shelters introduce untreated surfaces. Such situations can be countered only by multiple short-cycle applications. Increased humidity in houses had been known to increase the activity of chlorinated hydrocarbon deposits.

It is highly desirable that the final selection of the insecticide, dosage, and cycle be preceded by preliminary investigations in which all the above factors can be evaluated.

During this period, the possibility of reducing the coverage without loss of over-all effectiveness should be explored, taking into account the habits of the mosquitos. These possibilities include the omission of outbuildings and animal sheds, or not treating the lower or upper portions of walls or the interior of peaked roofs. Although complete coverage of the interior surface of a dwelling provides maximum protection, a reduction in the surface area to be sprayed affords economies in both manpower and insecticides.

In the USSR, it has been shown that during malaria eradication activities in the Azerbaijan Republic, control of malaria vectors was obtained even in rooms where larvae of silkworm were maintained. This shows that with the proper dosage of DDT emulsion sprayed in such rooms, no harm to the silkworm industry need be caused.

Space sprays containing pyrethrin extracts have in the past yielded very satisfactory control of malaria in certain areas where the vector species

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[1] Bio-assay kits are available from the World Health Organization, Vector Control Unit, Division of Environmental Health, Geneva, Switzerland.

was predominantly endophilic. Today, such space sprays cannot compete with the residual sprays as regards either effectiveness or cost, but in situations where immediate destruction of large numbers of adult mosquitos is necessary for disease control or for abatement of nuisance, they are still useful. The spraying may be done with mist-producing hand or power sprayers, with fog generators, or by aerial spraying. In closed spaces, a final spray mixture containing 0.2% of pyrethrins can be used at the rate of 1 g per m^3 (1 oz per 1000 ft^3).

3.1.2 *Larvicides*

Larval control in antimalaria campaigns has limited application at present. For anopheline larvae, DDT can be used in a solution containing 6 g of DDT per litre (0.05 lb per US gal or 0.06 lb per Imp gal) of fuel oil, hand-sprayed at the rate of 9.4 litres per ha (1 gal per acre); it can also be applied as a finished emulsion prepared with a few ml of an efficient solvent-emulsifier combination at the same DDT concentration, or a 20% solution in methylated naphthalenes—such as Velsicol NR-70 or Sovacide 544B—may be sprayed by aircraft at the rate of 56-112 g of DDT per ha (0.05-0.1 lb per acre). When ground apparatus other than a hand-sprayer is used, the dosage rate must be adjusted according to the type of equipment employed. If a species shows signs of resistance to DDT, it is possible to substitute heptachlor, dieldrin, lindane, or chlordane at the rate of 112 g per ha (0.1 lb per acre), or malathion at 225-450 g per ha (0.2-0.4 lb per acre). New organophosphorus compounds and carbamates are under study as mosquito larvicides. To penetrate heavy vegetative growth over watercourses, granular or pellet preparations of the toxicant are recommended. Control of *A. gambiae* persisting for four months was obtained in East Africa with 2% dieldrin granules applied as a residual larvicide at the rate of 1 lb of dieldrin per acre (1120 g per ha).

Where the water is difficult to treat adequately, or when it contains considerable organic matter, these rates may be increased substantially, provided the safety of fish and other wildlife is not at stake. Conventional methods of control, such as the application of oil, may still be used if desired.

In larval control, attention should be directed towards the elimination of man-made breeding places, such as wheel ruts, burrow pits, obstructed streams, irrigation ditches, and artificial collections of standing or impounded water. Wheel ruts, burrow pits and small swamps should be filled whenever possible. If permanent fills are not possible, drainage may be utilized. Where there is considerable fluctuation in stream levels, channelling to concentrate water flow, manipulation of the water level to strand eggs and larvae, or the construction of dams equipped with automatic siphons or hand-operated sluices to flush out breeding areas will help in mosquito control. The removal of vegetation from the edge of a stream is

an important control measure. Weed killers may be of use here. All these measures should be considered important for anopheline control.

In El Salvador, resistance to chlorinated hydrocarbons in the local anophelines has caused the return to Paris green dust applications on a trial basis.

3.2 Culicines

Many culicines are disease vectors, and many others demand extensive control because of the annoyance they cause, even though they may not carry disease. Extensive larvicidal treatments with synthetic insecticides have frequently led to the development of resistance and resulted in the use of an increasing variety of insecticides. Permanent control of culicines may be best accomplished by measures aimed at reduction of breeding areas. Insecticides should be applied as an adjunct when necessary for the protection of health and relief from mosquito annoyance. Measures aimed at reduction of breeding areas are considered under larvicides.

3.2.1 *Larvicides*

Control of culicine mosquitos should include measures directed at the elimination of larval breeding, including permanent filling of breeding areas, drainage by ditching, and impoundment or flooding of breeding areas. Efficient irrigation practices can reduce or eliminate some of the worst culicine problems. Cleaning out vegetation in water storage areas will reduce or prevent breeding. Elimination of discarded containers or tires is essential.

A large number of insecticides have been developed for use as larvicides against culicine mosquitos. Where resistance to one or more of these has occurred, substitution of another compound must, of course, be made. Larvicides may be applied by hand- or power-sprayers, or sprayed from aircraft as emulsions prepared from emulsion concentrates, solutions in oil, or wettable powders, or they may be used as granules or pellets. In larvicide applications, the degree of control obtained is frequently dependent upon the degree of pollution and the type and amount of vegetative cover. When cover is heavy it may be necessary to increase the dosage or the amount dispersed. Under these conditions, granules provide better results as they penetrate the cover more easily. The effectiveness of residual larvicides is dependent upon the larvicide itself, the stability of the water at the site, and the degree of pollution. Where water percolates rapidly through soil or is heavily polluted, a residual insecticide may become ineffective within a few days.

Susceptible populations of culicines can be controlled by DDT (56-448 g per ha ; 0.05-0.4 lb per acre) or by dieldrin, chlordane, heptachlor or lindane (112 g per ha ; 0.1 lb per acre). For mosquito populations resistant

to these compounds, organophosphorus insecticides are chiefly employed. Malathion (224-560 g per ha ; 0.2-0.5 lb per acre), fenthion (28-56 g per ha ; 0.025-0.05 lb per acre), parathion or parathion-methyl (56-112 g per ha ; 0.05-0.1 lb per acre), and EPN (45-84 g per ha ; 0.04-0.075 lb per acre) are effective larvicides. With sprays applied either from the ground or by aircraft, the amount of finished spray applied per acre varies from 1 quart to 10 quarts depending on the concentration and the equipment used. The weight of granular formulations applied per acre also depends on the concentration of insecticide in the granules and the size of the granules themselves. A common application rate is 10 lb per acre (11.0 kg per ha). In certain areas DDT, dieldrin, lindane and heptachlor have been used as residual larvicides or pre-hatch treatments. When used in this way, the dosage is increased to 1, 2 or more pounds per acre. These treatments are harmful to fish and wildlife and should not be used in areas where they may cause damage. In areas of chlorinated hydrocarbon resistance in the south-eastern USA, a granular formulation of Paris green admixed with vermiculite oil and granite dust, has been used fairly extensively but has proved expensive.

Since *Culex pipiens fatigans (Culex quinquefasciatus)*, a major transmitter of filariasis, is not as susceptible to DDT and is frequently found in polluted water, the dosage of DDT and other chlorinated insecticides (to which resistance develops rapidly) may have to be two to three times that usually employed to obtain satisfactory control of this species. In certain tropical areas, e.g., Malaya, the only reliable larvicides are certain unrefined oils, such as Malariol, with spreading pressures not less than 20 dynes per cm, applied at the heavy rate of 45-60 litres per ha (30-40 US gal or 24-32 Imp gal per acre). Effective control, but at greater cost, may be obtained with pyrethrum emulsions prepared by the dilution of a 20% concentrate to 0.1% or less.

The control of *Mansonia* mosquitos, the main vectors of rural filariasis in Ceylon, has been achieved by the application of the sodium salt of methylchlorophenoxyacetic acid as a herbicide against the host plant *Pistia stratiotes*. An aqueous solution containing 12.5 g of the herbicide per litre (2 oz per Imp gal) is sprayed evenly over the *Pistia*-infested area ; about 400 litres per ha (36 Imp gal or 43 US gal per acre) are required. Young plants are killed in 5-10 days and old plants within about 14 days.

For treatment of artificial water containers, crab holes and tree holes, the use of dieldrin cement pellets offers promise. Incorporation of one part of dieldrin water-dispersible powder (50%) in two parts of a sand/cement mixture (5 : 1) and application at the rate of one 10-g pellet to 9.0 litres (2.4 US gal or 2 Imp gal) of water has kept water jugs free from larval infestation for as long as one year. The low level of solubility of dieldrin in water (less than 0.1 p.p.m.) suggests a minimum toxic hazard to mammals, but further studies are required on this aspect.

As larvicides, oil or gasoline or a combination of these two has been used effectively. Pyrethrins applied in oil solution have been used for control when the concentration of spray was 0.006% (516 litres per ha ; 55 US gal per acre) or 0.07% (140-235 litres per ha ; 15-25 US gal per acre).

3.2.2 *Residual treatments*

Residual sprays are useful in controlling mosquitos indoors and are also of value in reducing numbers of mosquitos when applied to vegetation around habitations or breeding areas. They should be applied with equipment and methods of application discussed under anophelines. *Culex quinquefasciatus (C. fatigans)*, a major transmitter of filariasis, is not as susceptible to DDT on initial exposure as are the anophelines : moreover, it readily develops resistance to DDT and strong resistance to dieldrin and lindane.

Spraying of vegetation outdoors or around breeding areas with large amounts of oil sprays will injure or kill plants and should be eschewed. In the USA restrictions are applied to the use of insecticides in areas where food or feed crops are grown ; range or pasture, and food or feed crops themselves should not be treated. In homes, some residual sprays may be used for spot treatment. All insecticides should be used with care where fish and wildlife may be endangered, either directly or from drainage of treated waters.

Special note on perifocal treatment for Aedes aegypti *eradication*

In the Americas, *Aedes aegypti* is a domesticated species ; no truly feral race has ever been discovered. Furthermore, it has a limited dispersal capacity—usually less than 100 m (110 yd). These two factors greatly facilitate the eradication operations. However, the effectiveness of eradication operations depends upon the thoroughness of treatment in an area and upon adequate surveillance to detect and eliminate breeding foci.

Because, in the Americas, the objective is the eradication of *Aedes aegypti*, the method used is discussed separately in these recommendations. Eradication programmes are based chiefly on the " perifocal " treatment of all potential breeding places with a residual insecticide, i.e., the spraying of the inside and outside of containers plus the treatment of immediately adjacent wall surfaces. The insecticide of choice is DDT applied as a 5% suspension prepared from a water-dispersible powder. Physiological resistance to DDT has been observed in the Caribbean area. In areas in which resistance to DDT has been confirmed, lindane or dieldrin may be used. These two insecticides, as well as DDT, may be applied using the same equipment, spray concentrations, and rates of application as described under residual treatments for anophelines.

The " perifocal " treatment is repeated as many times as may be necessary to free a community of *A. aegypti*. The results of the eradication programme are assessed by surveys for larvae or adults at predetermined intervals corresponding to the times of the year at which breeding would normally occur. When three consecutive comprehensive surveys made during a period of not less than a year fail to find *A. aegypti*, the programme is considered successful.

For the treatment of some types of large artificial water containers and tree holes, the use of dieldrin-cement pellets appears to offer promise. The preparation and application of these pellets are described under larvicides for culicines, section 3.2.1. The low level of solubility of dieldrin in water (less than 0.1 p.p.m.) suggests that the toxic hazard to domestic animals and man is negligible, but further studies are needed. Residual spraying carried out for malaria control or eradication has a marked effect on any susceptible *A. aegypti* that may be present in the area. This should be taken into consideration when planning an *A. aegypti* eradication campaign.

In certain yellow fever receptive seaports and airports of Africa and Asia, control measures for *A. aegypti* are mandatory under the International Sanitary Regulations and are conducted primarily by means of larvicides supplemented by source reduction.

3.2.3 *Space sprays*

Space sprays for mosquito control range from the use of aerosol dispensers in homes, through the use of ground equipment for dispersing mists, fogs or dusts, to aircraft application of sprays and fogs. All treatments are effective in reducing the numbers of mosquitos, but the reduction is, in general, only temporary, since the sprays have no residual action and kill only mosquitos with which they come in contact. Re-infestation from outside sources generally occurs within a short time. However, if sufficiently large areas are treated or re-infestation pressure is not great, space sprays may be effective for longer periods. The frequency of space treatments will be governed by the rate of re-infestation. The use of space sprays for adult control has been favoured in some areas since the selection for resistance may not be as great as that obtained with the use of larvicides.

For the control of adult mosquitos in dwelling places, space sprays of pyrethrins (0.25%) plus a synergist (2%), or of methoxychlor (3%), DDT (3-5%), or malathion (2%) are effective.

For the control of adults outdoors, fogs or mists applied from various types of powered ground equipment or from aeroplanes are effective. The compounds and dosages indicated in Table 4 have been found effective.

These recommendations are based on the weights (lb) of actual insecticide applied per acre. With ground equipment mounted on moving vehicles the operator must know the concentration of the formulation, the rate

TABLE 4. RECOMMENDED DOSAGES OF INSECTICIDE SPRAYS FOR MOSQUITO CONTROL

Insecticide	g per ha	lb per acre	Remarks
DDT	224	0.2	Can be used where resistance is not a problem.
Lindane	112-224	0.1-0.2	
Chlordane	224-448	0.2-0.4	
Heptachlor	112	0.1	
Malathion	112-560	0.1-0.5	Use where resistance to chlorinated hydrocarbons occurs. Malathion has been used extensively and effectively as a fog.
Dibrom	56-280	0.05-0.25	
Fenthion	112	0.1	
Dichlorvos	56-280	0.05-0.25	

of application, the effective swath width of the treatment and the speed of the vehicle. Swath widths of 200 to 300 ft usually give accurate dosage. The rate of application varies from 7-25 gal per mile. Concentrations of sprays vary from 5% to 20%. The speed of the vehicle should not exceed 5 miles per hour. Fogs require fewer gallons per mile than do sprays and are usually more effective than sprays or dusts, at least with the organophosphorus insecticides.

Airplane fogging with malathion, which is being developed experimentally, has been shown to be effective and a large area can be covered rapidly at low cost. 10% malathion in oil applied at the rate of 120 gal per hour gave effective control. In this manner 1200 acres were treated in approximately one hour using 100 gal of solution. This treatment thus covered 12 acres per gal, a dosage of 0.07 lb of malathion per acre.

With all space sprays, weather conditions have a pronounced effect on the efficacy of the treatment.

3.3 Repellents

The interest of mosquito repellents lies mainly in their use as skin applications. Experiments against various species of mosquitos in Panama and the USA including Alaska have shown diethyltoluamide to be outstanding as an all-purpose repellent. The meta-isomer is more effective than the ortho- and para-isomers, and should constitute at least 70% of the technical product. Chlordiethylbenzamide is also highly effective against a wide range of species, and ethylhexanediol, dimethyl phthalate, dimethyl carbate, and indalone are all good general repellents that are outstanding against certain species. The period of protection provided by any one of these repellents varies with the individual, the temperature, the amount

of activity that would induce sweating, the amount of rubbing the treated surface receives, and the avidity of the insects.

All these repellents must be used with caution as they will damage such plastic materials as watch-crystals and fountain pens, some types of rayon and other synthetic fabrics (but not nylon), and articles that are painted or varnished. They will not damage cotton or wool. Ethylhexanediol has the least injurious effect, and diethyltoluamide and chlordiethylbenzamide are less injurious than the other materials. Diethyltoluamide and chlordiethylbenzamide feel less oily on the skin than the other repellents, and diethyltoluamide is sufficiently effective to permit some dilution with alcohol, which increases its cosmetic acceptability still more.

TABLE 5. SUMMARY OF RECOMMENDED INSECTICIDES FOR MOSQUITO CONTROL

Insecticide	Formulation or dosage	Application
	Residual treatments — anophelines and culicines	
DDT	2 g/m² (200 mg/ft²)	Apply with stirrup pumps or compressed air, hand or power sprayers to interior of houses and other buildings. See Table 10, page 218 for spray concentrations and application rates.
Dieldrin	0.6 g/m² (60 mg/ft²)	
Lindane	0.5 g/m² (50 mg/ft²)	
Malathion	2 g/m² (200 mg/ft²)	
	Residual treatments — culicines only	
Chlordane	1 g/m² (100 mg/ft²)	
	Larvicides	
DDT	56-448 g/ha ; 0.05-0.4 lb/acre	Apply as sprays (emulsions, wettable powders, or solutions) by hand or power equipment or from aircraft, or use as granules.
Dieldrin	112 g/ha ; 0.1 lb/acre	
Lindane	112 g/ha ; 0.1 lb/acre	
Chlordane	112 g/ha ; 0.1 lb/acre	
Heptachlor	112 g/ha ; 0.1 lb/acre	
Malathion	224-560 g/ha ; 0.2-0.5 lb/acre	
Parathion	56-112 g/ha ; 0.05-0.1 lb/acre	
Parathion-methyl	56-112 g/ha ; 0.05-0.1 lb/acre	
Fenthion	28-56 g/ha ; 0.025-0.05 lb/acre	
EPN	45-84 g/ha ; 0.04-0.075 lb/acre	
	Space sprays	
DDT	224 g/ha ; 0.2 lb/acre	Can be applied from ground equipment or aircraft. See text for application with different equipment.
Lindane	112-224 g/ha ; 0.1-0.2 lb/acre	
Chlordane	224-448 g/ha ; 0.2-0.4 lb/acre	
Heptachlor	112 g/ha ; 0.1 lb/acre	
Malathion	112-560 g/ha ; 0.1-0.5 lb/acre	
Dibrom	56-280 g/ha ; 0.05-0.25 lb/acre	
Fenthion	112 g/ha ; 0.1 lb/acre	
Dichlorvos	56-280 g/ha ; 0.05-0.25 lb/acre	

Repellents suitable for application to clothing are required by troops or other persons exposed to heavy mosquito infestations, since mosquitos bite readily through untreated clothing of normal weight. The treatment of clothes does not eliminate the need for skin applications as none of the repellents has enough spatial action to protect the exposed hands and face when applied to the clothing only.

All the repellents that are suitable for use on the skin may also be applied to clothing (except certain synthetic fabrics such as rayon), but some of the best clothing treatments are not recommended for skin applications. The most effective repellents recommended for the treatment of clothing are butylethylpropanediol and diethyltoluamide. The former should not be applied directly to the skin. The repellent can be applied as a spray, or by impregnating the clothing in the manner described in section 6.2 for chigger repellents.

4. HUMAN LICE

Three kinds of lice infest man—body lice *(Pediculus humanus)*, head lice *(Pediculus humanus capitis)* and crab lice *(Phthirus pubis)*. Since the body louse is the vector of epidemic typhus and relapsing fever, the development of effective methods of control for this species has received the most attention. Although head lice and crab lice are not important in disease transmission, they are annoying pests which can be controlled.

4.1 Body lice

Body lice have developed resistance to DDT and lindane in many different parts of the world. Before a control programme is begun and at six-monthly intervals during the programme the level of susceptibility of the population should be tested to ensure the selection of the proper insecticide and to determine whether resistance is developing.

The most satisfactory type of formulation for delousing treatments is a powder (pyrophyllite or talc) containing the insecticide because it can be applied easily and rapidly. It can be distributed in sifter-top cans (about 50 g or $1^3/_4$ oz capacity) for individual use, or applied to large groups with compressed-air dusters. With the sifter-top can, 30 g (1 oz) of an insecticidal powder are required for one person. The powder is applied over the inner surface of the underwear, with special attention to the seams, and spread evenly by hand. The seams and folds inside the other garments should also be treated, as well as the socks. If it is impossible to remove the clothing, the powder may be shaken into it through the openings. Mass treatments using hand-operated dusters or motor-driven air compressors with as many as 10 duster heads have given good control of lice and/or typhus

in large groups of people. With this type of treatment the clothing is not removed and 50 g ($1^3/_4$ oz) are required for one person. The powder is blown down the neck of the shirt, up the sleeves, and into the loosened trousers from several angles at the front and back. In delousing women an extra quantity may be blown down the neck of the dress and application at the waistline omitted. In all cases, the hair, head covering, extra clothing and bedding should be treated.

Where DDT-resistant lice are not encountered, a powder containing 10% of DDT is the preferred treatment. A single application will eradicate an infestation. Although DDT is not ovicidal, its long residual action will kill nymphs that hatch from eggs, usually in about two weeks. Where DDT-resistant lice are encountered, a powder containing 1% of malathion is effective. It is applied in the same way as DDT powder, but since it is not so long lasting a second application within 3-4 weeks may be necessary. It is ovicidal and residual, and a single application is sufficient. Extensive tests in the USA, Korea and Egypt have proved this treatment to be safe and effective. A powder containing 1% lindane may be used if resistance to both DDT and malathion is encountered. It should be applied in the same manner as DDT powder and a second application within 7-10 days may be necessary.

Powders containing synergized pyrethrum or allethrin are satisfactory for individual use, but less suitable than DDT, malathion or lindane for mass treatment. Because of their oily ingredients, the powders tend to clog the duster and do not sift throughout the entire clothing. One formulation that has been effective in practical use contains 0.2% of pyrethrins, 2% of sulfoxide (as a synergist), 2% of 2,4-dinitroanisole (as an ovicide), 0.1% of phenol S (isopropyl cresols, as an antioxidant), and 3% of a conditioner. Other synergists may be used in place of sulfoxide. This powder causes a rapid reduction in the number of lice, but has a shorter residual action than DDT ; treatments should therefore be repeated each week until the infestation is controlled.

In eastern Europe, good control has been obtained by the following methods : (1) soaking clothes in four times their weight of 1% DDT emulsion, (2) brushing 5% DDT emulsion into clothes to give 2 g of DDT per m^2 of clothing, (3) cross-hatching clothes with wax crayons containing 30% paraffin wax, 63% DDT, and 7% lindane, (4) distributing to each infested family a 500-g cake of soap containing 3% DDT, sufficient for washing the person and clothes for two weeks, and (5) washing clothes with 7% DDT soaps. The use of impregnated linen clothing was also tried but resulted in the development of DDT resistance after one year to such an extent that control could no longer be achieved.

Autoclaving of clothes in steam sterilizers or their fumigation with methyl bromide at 1 lb per 1000 ft^3 may be carried out at hygiene centres for body-louse control.

4.2 Head and crab lice

Although suspected cases of DDT resistance in head and crab lice have been reported, none has been confirmed. These lice live continuously on the head and body and attach their eggs to hairs. Consequently, treatments must be applied to infested parts. An emulsion concentrate designated NBIN (68% benzyl benzoate, 6% DDT, 12% benzocaine, and 14% Tween 80)[1] is highly effective against both species. Another effective emulsion concentrate is 1% lindane in alcohol together with an emulsifier. For application, 1 part of the concentrate is diluted with 5 parts of water, the hair is wetted thoroughly, and the emulsion sprayed or sponged on the infested parts. This treatment kills eggs as well as active stages. Treated persons should not bathe or shampoo for at least 24 hours.

The powder treatments recommended for the control of body lice, with the possible exception of malathion powder which has not been tested, are also effective against head and crab lice. The pyrethrum powder stops activity of lice very quickly. However, powder treatments are generally unpopular for several reasons. They make the hair chalky white and advertise the fact that the individual has lice ; consequently, he is tempted to wash the powder out as soon as possible, making the treatment ineffective. DDT powder causes the lice to become abnormally active and intensely irritating for a short time. Powders should be applied at weekly intervals if the hair is washed between treatments.

When there is a preference for oil formulations, as for example in South-East Asia, 0.2% lindane dissolved in coconut oil or a similar carrier has been found to be effective. In eastern Europe, tinctures of derris roots (Derritol) and delphinium flowers (Delfinol) have been found satisfactory for schoolchildren.

5. TICKS

Ticks hold a peculiar position among arthropod vectors of disease, many of the tick-borne diseases being transmitted from generation to generation through the eggs (transovarian transmission). Thus, ordinary control measures aimed at reducing numbers to a minimum may not be adequate to eliminate the disease from the human and animal populations.

5.1 Insecticides

Area control of ticks can be obtained with applications of any one of several chlorinated hydrocarbon insecticides ; DDT, chlordane, dieldrin and toxaphene, at rates of 1.12-2.24 kg of toxicant per ha (1-2 lb per acre), or lindane at the rate of 560 g per ha (0.5 lb per acre), give effective control

[1] Polyoxyalkalene derivative of sorbitan mono-oleate.

under field conditions. Sprays (suspensions or emulsions) or dust formulations of these pesticides produce similar results. Parathion also provides excellent immediate control. The level of control secured is dependent on the adequacy of the coverage : in brush areas, about 470 litres of spray or 45 kg of dust per ha (50 US gal or 42 Imp gal of spray, or 40 lb of dust per acre) are required, as compared with approximately half these amounts in sites of thin cover. Treatment with these chemicals usually prevents re-infestation for 30 days or more.

Generally, a thorough knowledge of the habits of a given species of tick must be obtained in order to plan an effective control programme. Such knowledge often allows control measures to be applied to limited areas rather than to an entire countryside. For example, since *Dermacentor variabilis*, the American dog tick, congregates along roads and trails, effective control can be accomplished by restricting treatments to swaths 6-9 m (20-30 ft) wide on each side of them.

The habits of some ticks are such that changes in the environment may be expected to result in their local eradication. For example, *D. variabilis* feeds in its immature stages on certain species of mice. These mice are present in large numbers in abandoned meadows and pastures. Replanting the abandoned pastures to forests will eventually alter the ecological conditions so that these species of mice may disappear, and consequently the dog tick too. However, information on the ecology of ticks is not so complete for all species.

Control of ticks on animals presents several important problems. Although this method is sometimes more economical than controlling ticks on vegetation, its advantages are offset by the tendency for many of the toxicants used to be stored in the fat or secreted in the milk of dairy animals.

Ticks, principally the brown dog tick, *Rhipicephalus sanguineus*, occasionally invade dwellings and cause severe annoyance to the occupants. Dogs may be freed of ticks by treating them with insecticide. Washes are usually more effective than sprays or dusts, as they are better able to penetrate the hair and reach all the ticks. A wash or spray should contain one of the following : 1% of DDT, 0.5% of malathion, or 0.05% of lindane or rotenone. If a dip is preferred, one-half of these concentrations should be used and the animal immersed except for the head. If a dust is preferred, ready-to-use products containing 5% or 10% of DDT, 1% of lindane, 4% or 5% of malathion, or 3% to 5% of rotenone can be obtained. Successful control usually requires treatment of both the animal and the premises which it frequents.

Infestations in homes and kennels may be eradicated with formulations of 5% of DDT, 2% of chlordane or malathion, or 0.5% of lindane or dieldrin in deodorized kerosene. These formulations must not be applied directly on animals, but in rooms to which dogs have had access they should

be applied as a coarse spray or with a paint brush to woodwork, floor and wall cracks, behind pictures and draperies, and other possible hiding places of the tick. All surfaces in kennels, with particular attention to cracks and crevices, should be sprayed to the point of run-off. If ticks persist for more than three weeks, a second application should be made. In some areas of the southern USA infestations are sometimes encountered that are not susceptible to control by chlorinated hydrocarbons. Diazinon (0.5% emulsion or solution), malathion or fenthion (1% to 3% sprays) or Sevin (2% suspension) are used to treat indoor harbourage sites, as noted above, as well as fences, shrubs, yards and the exteriors of buildings.

Ornithodorus moubata, responsible for the transmission of tick-borne relapsing fever in Africa, is a house-dwelling arthropod which tends to spend the greater portion of its life in the vicinity of its hatching-place. Personal hygiene and strict sanitary measures play an important role in preventing re-infestation, and permanent control may be achieved by the construction of special tick-proof dwellings.

In British Somaliland, *O. moubata* was controlled successfully by applying 0.1-0.15 g of lindane per m^2 (10-15 mg per ft^2), in the form of a water-dispersible powder, to the floors and to the lower 30 cm (12 in) of interior wall surfaces of infested premises. Two applications 4-6 weeks apart were sufficient to achieve control. For complete elimination of *O. moubata* in houses in East Africa, however, two sprayings of BHC at the rate of 12.5 g of gamma isomer per m^2 (1250 mg per ft^2) were necessary.

An application of a 0.5% lindane dust to the floors and lower parts of walls at the rate of 150-200 g of dust per 10 m^2 (3-4 lb per 100 ft^2) has also been found to be effective, provided repeated applications at monthly intervals are made.

In South Africa, 6 g of lindane per m^2 (600 mg per ft^2) applied in well-constructed dwellings controlled *O. moubata* for up to 27 months; 3 g per m^2 (300 mg per ft^2) gave control for approximately 12 months.

In Fort Jameson, Northern Rhodesia, an economical way to achieve control was found : BHC powder is mixed with sawdust to give a preparation containing 5% (by weight) of the gamma-isomer, e.g., 2.5 kg (3.5 lb) of BHC (40% gamma-isomer) water-dispersible powder is mixed with 17.5 kg (43.8 lb) of sawdust. This mixture is laid as a 10-cm (4-inch) barrier at the base of all inside walls of houses. The mixture requires renewal at frequent intervals.

In the Middle East, the control of *O. tholozani*, a cave- or burrow-dwelling species, presents particular problems and no satisfactory measures have as yet been developed.

In the USSR, massive dusting of forests with 10% DDT dust at 30 kg and 50 kg of dust per ha (27 lb and 45 lb per acre) for ground and aerial applications, respectively, has been employed against *Ixodes persulcatus*, the chief vector of spring-summer encephalitis ; 90-99% control was achieved

in the first year and eradication in four years after the one treatment. *I. ricinus*, the vector in Czechoslovakia, is less susceptible to DDT; here 10% lindane dusts at 80 kg per ha (71 lb per acre) have given almost complete control for the entire year. Aerosol treatments of the grassy scrub areas with 40% lindane concentrates at 5 litres per ha ($^1/_2$ gal per acre), using a Swingfog generator, give the desired very high control only for the first part of the season. When using a 10% concentrate at the rate of 6-8 litres per ha, a decrease in the number of ticks could be observed, particularly within the first 10 days of treatment. Thereafter the number of ticks increased gradually, but a reduction in numbers was apparent on most treated areas during the whole observation period. It is necessary to apply the aerosol at the appropriate time and sometimes to repeat the application, especially after a rainy period, which annuls the effect of the aerosol. *Hyalomma plumbeum*, vector of haemorrhagic fever in Bulgaria, has been eliminated by 20% DDT dust applied at the rate of 20 kg of dust per ha (19 lb per acre).

5.2 Repellents

Tick repellents are most effective when applied to the clothing. The socks and all the outer clothing should be treated by the impregnation method described in section 6.2 on chigger repellents. None of the repellents provides complete protection against ticks, but several provide more than 90% protection through various periods of aging and wear. The best repellents now recommended for general use against ticks are indalone, diethyltoluamide, dimethyl carbate, dimethyl phthalate, and benzyl benzoate. Undecylenic (undecanoic) acid is more effective, but has a disagreeable odour. Butylacetanilide, propylacetanilide, and isopropylacetanilide are the most effective, but the first is not approved for general civilian use in the USA and the last two are not widely available commercially and are expensive.

6. CHIGGERS

Trombicula akamushi and *T. deliensis* are vectors of scrub typhus in Asia and in the South Pacific. Although not recorded as disease vectors in other areas, related species are well known for their extreme annoyance to man. Area treatment with insecticides and personal protection with repellents are the chief means of coping with chigger infestations.

6.1 Insecticides

In area treatment, the locality should be treated thoroughly with toxaphene or chlordane, at 2.25 kg per ha (2 lb per acre), lindane at 280-560 g per ha (0.25-0.5 lb per acre), or dieldrin at 560-1120 g per ha (0.5-1 lb

per acre). Either spray or dust formulations can be employed, special care being taken to ensure thorough coverage of the area. DDT is relatively ineffective against these mites.

6.2 Repellents

The materials used for personal protection against chiggers, or red bugs (the larvae of mites of *Trombicula* and related genera), function less as true repellents than as acaricides (mite toxicants), but may properly be considered in a discussion of repellents since the end result is the same. They are more effective when applied to the clothing than to the skin. The material now regarded as best for this purpose is benzyl benzoate; diethyltoluamide and the other mosquito repellents are effective while fresh and for certain periods of aging and wear after application to clothing, but benzyl benzoate also remains effective after the clothing has been washed. This may be important in the case of troops or other outdoor workers, who may be obliged to wade in streams or stay out in the rain. The clothing may be treated by any of the following methods:

(1) *Hand application.* The simplest way to treat the clothing is to pour about a dozen drops into one hand, rub the hands together, and then rub them lightly on the socks and other clothing. Make liberal applications along all openings, such as inside the neckband of shirts, the fly and turn-ups of trousers, and tops of socks. Do not apply benzyl benzoate by this method.

(2) *Spray method.* The material may be applied to the entire garment with a sprayer, with special attention to the openings (see barrier method below).

(3) *Barrier method.* Considerable protection will be obtained by treating only the openings of the clothes—inside the neckband, fly and cuffs of shirts; inside the waist-band, fly and turn-ups of trousers; and on the socks, both above and inside the shoes, below the tongue. Apply the material by daubing, spraying, or drawing the mouth of the bottle along the cloth to make a band 1.3 cm ($^1/_2$ inch) wide. Women's clothing may be protected in the same general way. Persons who will not be sitting or lying on the ground can obtain nearly complete protection by treating only the socks above the shoe tops and the bottoms of the trouser legs.

(4) *Impregnation method.* The best method of obtaining complete protection under all conditions of exposure is to impregnate all the outer clothing that will be worn in the field with a solution or emulsion of the repellent. A recommended impregnation rate is about 20 g of actual repellent per m^2 ($^1/_{15}$ oz per ft^2) of cloth, or a total of 70 g (2½ oz) for a jacket (or shirt), trousers, and socks of medium size. The underwear should not be treated.

A satisfactory solution can be made by dissolving the repellent in a volatile solvent, such as acetone or any dry-cleaning fluid. Each garment should be treated with enough liquid to wet it thoroughly, but no excess liquid should be left; usually about 1.5 litres (3 pints) of solution containing 70 g (2½ oz) of repellent will be required to treat an outfit of heavy cotton cloth. After all parts of the garment have been saturated, allow the solvent to evaporate.

An emulsion can be made by mixing 70 g (2½ oz) of the repellent with about 1.5 litres (3 pints) of water and 7 g (¼ oz) of an emulsifier, such as Tween 80 or Triton X-100, or 30 g (1 oz) of soap. Many synthetic household detergents are not suitable for making emulsions, but most laundry soaps are satisfactory. Dissolve the emulsifier or soap in the water and add the repellent slowly while stirring vigorously. If large quantities of clothing are to be treated, a stock solution containing 90% of the repellent and 10% of the emulsifier can be prepared and added to water, as needed, at the rate of 1 part in 16 (62 ml to 1 litre, or ½ pint to 1 gal). All parts of the garments should be saturated with the emulsion; they should then be wrung out lightly and *dried thoroughly* before being worn.

7. FLEAS

7.1 Oriental rat fleas

Xenopsylla cheopis, the principal transmitter of plague and endemic or murine typhus, has been readily controlled by the application of 5% or 10% DDT dust to rat runs and harbourage areas. While these two formulations are equally effective for *X. cheopis*, the greater toxicity of the 10% dust to other ectoparasites normally less susceptible to DDT, such as the cat flea, makes it the formulation of choice. Resistance to DDT has recently been confirmed in India. Laboratory tests have shown that malathion residues are effective in killing *X. cheopis*. Although confirmation of the effectiveness of malathion in the field has not yet been obtained, a 4% to 5% malathion dust should be effective in control.

In India there is evidence that indoor residual spraying and "patch dusting" have brought about marked reductions in flea density. In the indoor spraying DDT at a dosage of 2 g per m^2 (200 mg per ft^2) was used. With patch dusting, which is an easy, cheap and effective method, the dusting powder, which can be given to each householder with instructions for use, is deposited in small quantities under grain bins, on rat runs, and in other places where it is not likely to be disturbed. A later check by the programme supervisor will ensure that the work has been performed correctly. With this method, dieldrin (1.5%) and aldrin (2%) dusting powders gave zero flea indices four months after application. DDT (10%) powder gave an index of 0.3.

7.2 Dog and cat fleas

The dog and cat fleas, *Ctenocephalides canis* and *C. felis*, are widespread and abundant. They look alike and both attack dogs or cats as well as man. Although resistance to chlordane has not been confirmed by laboratory data, control failures in different parts of the world have led workers to assume the occurrence of resistance to this insecticide and to use other pesticides.

The successful control of these fleas depends on treatment of animals, animal quarters and premises. Satisfactory flea control on premises is largely dependent on the adequacy of coverage. Treatment of the animal and its quarters alone may often not eliminate an infestation, because the insect is also breeding at other sites on the premises. When these breeding sites remain untreated, the result is poor control.

Treatment of animals may be made with dusts, sprays or dips. Dip application should be made by a veterinarian. When treating an animal, care should be taken to prevent the insecticide from getting into its eyes and mouth. Chlordane, DDT and lindane should *not* be used on pups under two months old or on cats. The treatments in Table 6 are recommended.

TABLE 6. RECOMMENDED INSECTICIDES FOR DOG AND CAT FLEAS

Insecticide	Formulation [a]	Application
On animals		
Malathion	4% powder, 0.5% spray, 0.25% dip	Apply powder with a shaker-type dispenser, a puff duster, or any garden-type hand duster. Use about 1 tablespoon of powder on a medium-sized, short-haired dog, such as a fox terrier. Reduce or increase this amount according to size of animal and length of hair. Pay particular attention to back, neck and top of head. Rub powder thoroughly into hair. Do not use chlordane, DDT, or lindane on pups under 2 years of age or on cats. Sleeping quarters, kennels and other areas should be treated at the same time as the animal is disinfested.
Rotenone	1% powder	
Pyrethrum	1% powder	
Pyrethrins	0.2% plus 2% synergist as a powder	
Lindane	1% powder	
Methoxychlor	10% powder	
DDT	5% powder	
Chlordane	2-4% powder	
Sevin	2% powder	
On premises		
Diazinon	1% EC	Apply spray at the rate of 4-8 litres per 100 m² (1-2 gal per 1000 ft²) using compressed-air or power type sprayer.
Malathion	0.5% EC	
Lindane	1% EC	
Trichlorfon	1% EC	
Ronnel	1% EC	
Chlordane	1% EC	
DDT	5% EC	

[a] EC = emulsion concentrate

Sprays containing DDT, methoxychlor, malathion, or pyrethrum will destroy fleas in homes. Apply a 5% DDT or methoxychlor spray or a 2% malathion spray to floors, baseboards and walls to a height of about 1 ft. Apply a light mist to furniture upholstery, rugs and other fabrics. Commercially prepared household sprays containing pyrethrum may be applied in the same manner. Since these sprays usually contain low concentrations of insecticide, the treatment may have to be repeated in 7-10 days. Apply any of the sprays at the rate of about 1 quart to 250 ft^2. Use a hand sprayer, a household sprayer designed for treating surfaces, or a compressed-air sprayer. Apply a fine-mist spray, as a heavy spray may stain fabrics. Before spraying, it is preferable to clean rooms and upholstered furniture with a vacuum cleaner.

Special note on vectors of sylvatic plague

In the control of vectors of plague, attention has in the past centred mainly on the control of the oriental rat flea, *Xenopsylla cheopis*, and a few other closely-related species associated with domestic and semi-domestic rats. Sylvatic plague persists in large areas of a number of regions of the world. The permanent reservoir of *Pasteurella pestis* in these areas is maintained in different kinds of wild rodents, such as ground squirrels and the gerbil. Some species of fleas other than those on commensal domestic rodents are associated with the wild-rodent reservoirs of plague. Outbreaks of plague are frequently traced to reservoirs in wild rodents. Because of this and the possibility of increased contact between humans and wild rodent reservoirs, as well as the mingling of commensal and wild rodents as people move into rural or remote areas or use these areas to a greater extent for recreation, much interest and experimentation have been directed towards the control of vector species of fleas on wild rodents. Since the elimination of a focus of sylvatic plague by the destruction of wild rodents is not generally recommended, the control of fleas on these rodents may at times be necessary in areas of endemic plague.

There are many difficulties in controlling vector species of wild rodent fleas; the areas involved are large, are usually rather inaccessible and have sparse human populations. Recent experiments have demonstrated that the use of bait boxes containing DDT powder can control fleas on wild rodents and in their nests. About 50-90 g of a 5-10% DDT powder was placed on the floor boards of the bait boxes. While feeding, rodents picked up this powder and transported it to their nests. Because of the number of bait boxes required, this method would of necessity be restricted to very limited areas, such as those under agricultural use. Dusting of burrows is another possible method for controlling fleas of wild rodents. For the control of fleas on *Citellus* ground squirrels, dieldrin (2%) and aldrin (2.5%) dusts can be blown into burrows at about 30 g per burrow. The development of flea control should precede rodent control.

7.3 Repellents

Diethyltoluamide is an excellent repellent for fleas, particularly when used to impregnate socks and outer garments as described in section 6.2 under chigger repellents (page 192). Clothing impregnated with diethyltoluamide repels fleas for more than one week. Temporary protection can be obtained by smearing the repellent on the socks and trouser legs. Undecylenic (undecanoic) acid, propylacetanilide, and benzyl benzoate are also good flea repellents; clothing treated with these compounds remains effective for several days of ordinary wear.

8. COCKROACHES

Cockroaches are annoying pests and may spread disease by polluting food with filth carried on their legs and bodies. They destroy food and damage fabrics and book bindings. Roaches enter premises from outdoors, in infested containers, or from adjoining homes or apartments. To keep them out, all cracks, especially those around water and steam pipes entering rooms, should be filled with putty or plastic wood. Good housekeeping is the key to cockroach control; insecticides alone give only temporary relief. Keep all areas thoroughly clean so that no food particles, debris, dust and rubbish remain to foster cockroach infestation.

Infestations of cockroaches can be controlled with insecticidal sprays, dusts, lacquers, aerosols or baits. However, resistance to chlorinated hydrocarbons is present in many populations of the German cockroach, *Blattella germanica*. Where resistance occurs, control can be accomplished with organophosphorus insecticides or other compounds. No confirmed cases of resistance have been reported in *Periplaneta* spp.; chlorinated hydrocarbons are preferred for these species because of their long residual activity.

Kerosene solutions are less apt to injure fabrics and finishes on furniture but aqueous emulsions give more effective residual deposits. A single residual treatment in the right places gives protection for several weeks. A dust or water spray may be used when there is danger from fire. Both a liquid and a dust may be used when cockroaches are abundant, difficult to control or firmly established—a dust can be blown and floated into areas inaccessible to sprays. Liquid sprays are applied with ordinary household plunger type sprayers, compressed-air sprayers, power sprayers, or other special equipment. A coarse mist rather than a fine one should be used so that it wets the surface instead of merely floating away. Enough spray should be applied to moisten surfaces thoroughly without running or dripping. A paint brush may also be used. A pin spray application is useful for penetrating deep cracks. A light, uniform film of dust may be applied with a puff duster of the bulb, plunger, or bellows type; this

method of application is suitable for hiding places and surfaces across which roaches run. Pyrethrum dusts or aerosols may be used to flush cockroaches from concealed hiding places. If moisture causes caking, the treatment should be repeated. A band of dust around the edge of a room is not an adequate treatment. Aerosols should be directed into hiding places and give better results if all openings are afterwards closed to prevent roaches from escaping.

With all toxicants, treatment is made to restricted areas, the pesticide being applied to runways, cracks and crevices, behind and along baseboards, waterpipes and other harbourage areas. A simple way to find hiding places is to enter a dark room quietly, turn on the lights and watch where the cockroaches run. With chlorinated hydrocarbons, treatment of restricted areas, comprising about 20% of the total area of a room, has been found adequate. With the organophosphorus compounds a more thorough treatment of hiding areas and runways is needed.

Where resistance is not encountered, chlordane and dieldrin are highly effective against all cockroaches and are the insecticides of choice. The formulations in Table 7 are recommended for cockroach control.

For highly resistant populations, the use of sodium fluoride (50% dust) has been reintroduced, but it is a dangerous material and less effective than the organophosphorus compounds. A combination of malathion (1 part) and Perthane (2 parts) is also suitable for control of resistant roaches when used at a 4% level. To obtain a quick kill in heavy roach infestations or to drive the insects from protected recesses, aerosol formulations, containing either pyrethrum or pyrethrum plus a chlorinated hydrocarbon, may be used alone or in combination with a residual treatment. When the combined application is made, irritation by pyrethrum activates the roaches so that they " pick up " a greater amount of the residual deposit.

TABLE 7. RECOMMENDED PESTICIDES FOR COCKROACH CONTROL

Insecticide	Solution or emulsion	Dust
Chlordane	2-2.5%	—
Dieldrin	0.5%	1%
Diazinon [a]	0.5-1.0%	2-5%
Malathion	1-3%	5%
Ronnel	2%	—
Fenthion [a]	3%	—
Dicapthon [a]	1%	—
Chlorthion [a]	1-2%	—
Trichlorfon [a]	1-2%	—
Lindane	0.5%	1%

[a] Should be used only by experienced personnel or pest control operators.

In the USA, a pelleted bait containing 0.125% of Kepone is now widely used to supplement residual insecticides in German roach control; it is also used as the sole control measure in special situations. The pellets are placed in small trays or scattered in concealed locations. This method of control, although slow, has been particularly useful in animal rooms, zoos, pet shops and in or about insectaries, where the use of conventional insecticides would jeopardize experimental animals or cultures. German roaches, brownband roaches, *Supella supellectilium* and *Periplaneta* have been controlled by this method.

9. BEDBUGS

Bedbugs feed on blood—principally that of man. They are spread chiefly by the clothing and baggage of travellers and visitors, by second-hand beds, bedding and furniture, and by laundry. Bedbugs may also be serious pests in animal and poultry houses and in laboratories where rabbits, rats, guinea-pigs or birds are kept for experimental purposes. They may also feed on small animals and birds kept as pets. The loss of blood may weaken these animals. Hiding places of bedbugs are usually made evident by black or brown spots of dried excrement on surfaces on which the bugs rest and by eggs, egg-shells and cast skins near these places. At the start of an infestation, bedbugs are usually found only about the tufts, seams and folds of mattresses and day-bed covers. Later they spread to crevices in bedsteads and eventually, if uncontrolled, behind base-boards, window and door casings, pictures and picture mouldings, and in furniture, loosened wall paper, cracks in plaster, and partitions.

Applying insecticides directly into hiding places will control bedbugs. Sprays are preferred to dusts since the latter do not cling to mattresses, bedsteads or vertical surfaces as well as sprays. Dusts are also more difficult to apply properly and are more unsightly than sprays. The effectiveness of control depends on the thoroughness with which the spray is applied. To treat a bed, enough spray to wet the slats, springs and frame should be applied without missing any crevices where bugs hide. A light mist is applied to the entire mattress (see precautions below), penetrating seams, tufts and folds. The bed should be allowed to dry for one or two hours before putting on sheets or occupying it. Upholstered furniture should be sprayed thoroughly (see precautions below). In treating other hiding places, enough spray should be applied to wet all surfaces to the point of run-off and to penetrate all crevices. Walls should be sprayed to a height of several feet above the floors.

In many areas bedbugs have developed resistance to DDT and in some localities the bugs have developed strong resistance to dieldrin and a lower resistance to lindane. Where resistance to DDT is not encountered, this is the insecticide of choice and a 5% DDT emulsion or solution should be

used. Lindane is also effective as a 0.1% spray on mattresses and upholstery and as a 0.5% spray in hiding places. However, diazinon as a 0.5% spray and malathion as a 0.5-1.0% spray are preferred to lindane, as they have a longer residual action. Ronnel as a 1% spray is also effective. The treatment of mattresses and upholstery with these insecticides must be done with care, giving only a light application of the toxicant. Under no circumstances should the treated materials be soaked with spray. As an additional precaution, bedding used by infants should not be treated.

Thorough applications of synergized pyrethrum sprays (0.2% pyrethrum and 2% synergist) give effective control. However, there is no residual activity, and retreatments one or two weeks apart may be required.

10. TRIATOMID BUGS

Species of *Triatoma*, *Panstrongylus* and *Rhodnius* (sub-family Triatomidae), also known as cone-nose bugs, assassin bugs, china bedbugs or kissing bugs, are important vectors of Chagas' disease in Mexico and Central and South America. The bugs live exclusively on the blood of animals, including man, and transmit *Trypanosoma cruzi*, the causative organism of Chagas' disease. In some other areas, where the disease has not been reported in humans, they frequently attack humans and their bites may cause intense itching, nausea, flushed face, palpitation of the heart, rapid breathing and pulse, and sometimes hives in sensitive persons.

These bugs spend most of their time in cracks, fissures and other hiding places in walls and ceilings of human dwellings and in animal habitations and nests. They emerge only to feed and then retreat into their hiding places. Where housing is primitive, conditions may be ideal for these bugs. The nests of animals, such as wood rats (*Neotoma*) or pack rats, etc., in California, around dwellings may be a source of these bugs. They are relatively long-living and can survive for long periods of time without a blood-meal.

The trypanosome responsible for Chagas' disease is not confined to humans, but is found in a wide range of animals. The distribution in this animal reservoir is much greater than that in humans, for it is found as far north as Texas, New Mexico and California, even though no human cases have been reported. In South America, very high natural infection rates have been found in *Triatoma* bugs. These bugs are not only important as disease transmitters but if present in large numbers their bites cause considerable annoyance and may even have debilitating effects.

The method of choice for the permanent control of *Triatoma* is the elimination of breeding or hiding places, including provision for improved dwellings and the clean-up or removal of animal nests around or under dwellings. Insecticides should be regarded as supplementary to this procedure. To date, DDT has not proved satisfactory for control of these

bugs; however, dieldrin and BHC (12% gamma isomer) have given good results in some localities. Satisfactory control has been obtained by the residual spraying of infested houses at the rate of 0.5 g gamma-isomer per m^2 (50 mg per ft^2) for BHC and 1.25 g per m^2 (125 mg per ft^2) for dieldrin (see houseflies, section 2.1.2, or anophelines, section 3.1.1, for equipment and methods of applying residual sprays).

11. VENOMOUS ARTHROPODS

Although venomous arthropods such as scorpions, wasps and spiders are not disease vectors, a section on their control is included because they constitute an important problem to those engaged in controlling insects of public health importance.

11.1 Scorpions

Recognition of scorpions is made easy by their more or less crab-like appearance and long, fleshy, segmented tail-like postabdomen ending in a bulbous sac and stinger. Scorpions are nocturnal, hiding during the day beneath loose stones or bark or fallen trees, boards, piles of lumber, accumulations of debris or stored household effects in basements or places adjacent to houses. Their stinger is normally used to paralyse insects, spiders or other prey upon which they feed. The sting of most scorpions is quite painful, but no more to be feared than the sting of a wasp; however, the sting of some scorpions is serious and can be fatal, particularly in young children or elderly people. If at all in doubt, the victim of a scorpion bite should be taken to a physician, particularly if he is under five years of age, if he has a heart ailment, or if he has been stung in many places, or on the face, neck or genitalia. Antiscorpion serum is available for most dangerous scorpions, but must be administered to the victim soon after he has been stung.

The risk of scorpion stings can be greatly reduced by eliminating their hiding places as mentioned above. In an area where scorpions are abundant, clothing should be shaken out and shoes inspected before dressing. Control can be obtained with spray solutions or emulsions containing 5% deodorized malathion (2 g per m^2; 200 mg per ft^2), 0.5% lindane (0.2 g per m^2; 20 mg per ft^2), or 0.5% dieldrin (0.2 g per m^2; 20 mg per ft^2), or with dusts containing 1% dieldrin or 1% lindane. In homes, these preparations should be applied to baseboards, mouldings, the underneaths of sinks, and areas around cracks and corners. Outside foundations, joists and floor supports, as well as the ground and rough outbuildings should also be treated.

In Brazil, a campaign was initiated in 1951 against the scorpion *Tityus serrulatus* with BHC applied at the rate of 0.5 g of the gamma isomer per m^2 (50 mg per ft^2). The treatment covered not only the interior walls of the

houses, but also the attics, back yards and brick walls around the houses. Stacks of wood and bricks, wherever located, were removed, the living scorpions were collected for serum production, and the place was treated with insecticide. Vacant lots were also covered with BHC. Once all houses in the city had been treated, control was maintained by spot treatments. Following the notification of scorpion stings or the presence of scorpions in houses, the corresponding city block was immediately sprayed.

11.2 Wasps

Wasps (yellow-jackets, hornets, *Polistes*, cicada killers) sting by driving a needle-like ovipositor into the flesh and injecting a venomous fluid into the wound. This causes a painful swelling that may last for several days. Occasional individuals show allergic reactions to insect venom which may cause severe general illness or death. If an allergic reaction is suspected, a physician should be consulted promptly.

Wasps may build nests in or around homes—beneath eaves, on porches or structural surfaces—or in trees, shrubbery, rock fences, and in holes in the ground. Most wasps kill destructive insects and are, therefore, beneficial. But when wasps build nests too close to the house or in shrubbery where children play, they should be destroyed.

Wasps can be controlled by applying an insecticidal spray or dust to their nest. Treatments should be made in the evening or early morning when the wasps are less active and most of them are in the nest. A 5% chlordane, 10% DDT, or 1% dieldrin dust applied to the opening of the nest can be used. Sprays, including oil solutions, emulsions, and wettable powders, of 2-2.5% chlordane, 5% DDT, 0.5% dieldrin, as well as combinations of lindane, DDT, methoxychlor, malathion, and pyrethrins are also effective. In Hong Kong, very good results were obtained with a kerosene-base spray containing 0.5% dieldrin, 0.08% pyrethrins and 0.63% piperonyl butoxide. Sprays should be directed towards the opening of nests and the nests sprayed until thoroughly wet. With a ground nest, a shovelful of moist earth thrown over the entrance after treatment will prevent dying wasps from gaining the surface. An underground nest can also be treated by pouring several ounces of carbon tetrachloride into the opening and covering with soil or absorbent cotton.

In the home, a 5% DDT, a 2% chlordane, or a 0.5% dieldrin oil spray can be used effectively to kill wasps. Dieldrin or chlordane should not be used for over-all spraying of the interior of the rooms; instead, screens, window frames and other places where wasps crawl should be treated. Individual wasps can be killed with a fly swatter or a direct spray from an aerosol bomb or household sprayer.

In Romania, good results have been obtained with toxic baits, including 0.1% trichlorfon, using melon pulp or other fruit as an attractant.

11.3 Spiders

Many kinds of spiders get into houses and other buildings and, when numerous, cause annoyance. Although most spiders are beneficial because they feed on other insects, some are dangerously poisonous to man. Most spiders avoid contact with man, but if they are accidentally touched or confined, many will attempt to bite in self-defence. Even when able to penetrate the skin, the venom that most spiders can inject is quite harmless. The bites of a few species can be dangerous, however, and medical attention should be sought for these.

Trash, piles of old lumber or brick, weeds, as well as woodsheds and similar structures, are good breeding places, and if they are near the house, many spiders may enter them. Spiders may also breed under buildings. The elimination or cleaning up of breeding places is an important step in control. Sprays made from emulsion concentrates, wettable powders, or solutions can be used to control spiders; however, oil-based sprays will injure vegetation and only emulsions or wettable powders should be used here. Sprays containing 2-3% chlordane, 0.2-0.5% lindane, 0.5% dieldrin, or 2% malathion applied to walls and webbing and along beams, in cracks and corners, under shelving or similar protected sites most frequented by spiders, are effective. They will kill spiders on contact and leave a residual deposit effective for two or three weeks. Caution should be used while spraying in closed places. Black widow spiders (*Latrodectus* spp.) irritated by the spray have been known to drop on to the person doing the spraying and bite him. Pyrethrum sprays will kill spiders on contact, but have no residual action. Creosote has been used successfully on the wood of outhouses to kill spiders and repel future invaders. Screens will keep larger spiders out; however, newly-hatched spiders are tiny and may enter through screens.

12. RODENTS

Sanitation is absolutely essential to the permanent control of commensal rats and mice; the use of rodenticides should be regarded as supplementary to sanitation. A programme of control should include: (1) rat-proofing of buildings, (2) elimination of harbourages, (3) elimination of food and water-supplies, and (4) destruction of the rodents themselves. Rat-proofing can be done at the time of construction with only a little extra cost, whereas rat-proofing of old buildings is usually expensive. Even when buildings have been rat-proofed, rats may gain access or be introduced in goods or cases. If sources of food and water are available, rats may breed prolifically. It is important to repair all leaks and otherwise remove sources of water. The proper storage of food so that it is inaccessible to rodents and the proper disposal of refuse or garbage from any source are essential. Cleanliness and efficient management greatly aid in the control of rats.

In the use of rodenticides a system of baiting is employed. Investigations have shown that yellow cornmeal is a readily acceptable and inexpensive bait material. Where it is not available, other grains or cereals, as well as bread crumbs, diced bread, raw meat and raw fish may be used. In some cases, a system of pre-baiting is used since rats are suspicious of new foods or objects. Offering a clean, poison-free bait for several days in selected places or containers before introducing the poisoned bait may be helpful. Under some circumstances, particularly where food is available to rodents at all times, e.g., in food warehouses, it may be difficult to induce rodents to take any bait. Such baiting problems are not usually related to the poison employed. A different food from the one readily available can be tried as bait, or aqueous solutions of the anticoagulant rodenticides, with 5% sugar as an attractant, will give good results if other sources of water are removed.

The anticoagulants have come into extensive use as rodenticides. Despite their widespread use in the USA there has not been any evidence of the development of resistance to them in the field in that country. However, " apparent resistance " to diphacinone (0.0025%) and to warfarin (0.005%) has been reported for Norway rat populations in a single farming area in Scotland.

The slow-acting, anticoagulant rodenticides—warfarin, pival, diphacinone and fumarin—and preferred for use in most situations because they are readily accepted by and effective against roof rats, Norway rats and most species of mice, and have a low toxic hazard to humans and domestic animals. The effect of the anticoagulants is cumulative and their rodenticidal action depends upon at least a small amount of the poison being consumed almost every day. To achieve effective control, the anticoagulant baits must be available to the rats for a period of at least one to two weeks. Establishment of permanent bait stations in places subject to reinfestation provides good continuous control.

Baiting for rodents needs to be carried out with fresh materials of the best quality. Frequent replacement of (not addition to) baits is important. Excess moisture may cause spoilage of grain baits. Such breakdown can be prevented by incorporating the anticoagulant bait in a paraffin base at a ratio of 1 : 1. Such semi-permanent baits have been reported effective against both rats and mice. It is often worthwhile to extend the baiting to the environs of properties being protected. This is most important around non-rat-proofed buildings used for food storage.

Table 8 gives recommendations for the use of anticoagulant rodenticides for the control of roof rats, *Rattus rattus*, Norway rats, *R. norvegicus*, and mice. Other rats in the *R. rattus* group, e.g., *R. rattus frugivorius*, *R. rattus rattus*, and *R. rattus alexandrinus* show a similar degree of susceptibility to these rodenticides, although fruit or other baits may be necessary in some parts of the world.

TABLE 8. ANTICOAGULANT RODENTICIDES FOR CONTROL OF RATS AND MICE

Rodenticide	Concentration [a] (p.p.m.) [b] of bait required for:		
	Roof rat	Norway rat	Mice
Warfarin [c]	250	50-250	250-500
Diphacinone [c]	50-100	50-100	125-250
Fumarin	250	250	250-500
Pival [c]	250	250	250-500

[a] Commercial preparations of anticoagulants generally contain 0.5% toxicant. The dilutions of 0.5% concentrate to give the recommended concentrations in p.p.m. are as follows:
500 p.p.m. — 1 part concentrate plus 9 parts bait
250 p.p.m. — 1 part concentrate plus 19 parts bait
100 p.p.m. — 1 part concentrate plus 49 parts bait
50 p.p.m. — 1 part concentrate plus 99 parts bait
[b] p.p.m. divided by 10 000 = % concentration.
[c] May be used as dry or liquid bait.

In addition to the anticoagulant materials, other rodenticides can be used with baits. Antu is not effective for the control of roof rats or mice. However, it still holds a definite place as a quick-acting poison for the Norway rat. The concentration generally employed in solid baits is 2-3%. Antu should not be used against the same populations more often than about once a year, for it induces a persistent bait shyness in rats. This property makes antu ineffective for repeated use against the same rat population. Its safety record is good and it may be used in residences and food-handling establishments.

Before the advent of the anticoagulants, red squill, incorporated in a meat, fish or cornmeal bait at a concentration of 10%, was the principal rodenticide. It continues to be of value in reducing populations of the Norway rat, but is ineffective against roof rats and mice. Since red squill acts as its own emetic in animals able to vomit, it is comparatively safe to use.

The rodenticide, sodium monofluoroacetate is still the most effective, fast-acting rodenticide for roof rats and Norway rats. However, its extreme toxicity to man and animals requires that it be used only by carefully trained crews for certain types of premises, and it must not be made available to the general public. It is used at a concentration of 12 g of sodium monofluoroacetate in 1 gal of water.

Thallium sulfate as a 0.5-1.5% bait and zinc phosphide as a 1-3% bait are also used as rodenticides. Zinc phosphide has an odour and taste objectionable to pets and man. Thallium sulfate has no distinctive odour or taste and is highly toxic, making it more dangerous to use. As it can be absorbed through unbroken skin, rubber gloves should be worn when handling or mixing it. The addition of tartar emetic (0.3-0.375%) in routine

poisoning is recommended. It is not recommended for general use but only by trained personnel. Unconsumed baits should be collected and safely disposed of. Corpses of animals killed with thallium sulfate should be disposed of in a safe manner by burying in the ground at least 50 cm deep and 10 m from water wells.

In controlling mice, it must be remembered that their movement in foraging for food is not great. The use of many well-distributed small baits is preferable to a few large ones. Because mice have a habit of nibbling when feeding, it is considered advisable to use relatively high dosages of anticoagulant to reduce the chances of operational control failures. Mice are not attracted to old bait, so frequent renewal is desirable. In experimental studies, the use of a 1% warfarin dust around water sources shows promise for mouse control in warehouses. DDT micronized powder (50%) is lethal to mice when applied to runways and harbourage areas.

13. BIRDS AND BATS

Research has demonstrated that wild birds of many species throughout the world serve as reservoirs for a variety of diseases that occasionally produce serious epidemics in humans. In addition to these reservoirs, it has been shown that soil contaminated by the droppings of bats and birds, particularly of starlings and pigeons and sometimes of wild birds and poultry, provides favourable environment for the development of fungi responsible for histoplasmosis and cryptococcosis. In some areas, the occurrence of these fungi in both man and animals has been shown to be widespread. Active control measures may have to be instituted against some birds under certain circumstances, e.g., where they create disease problems, cause annoyance and contamination, or around airports where they may be dangerous to aircraft.

Measures to control birds are very poorly developed, although certain species have been agricultural pests for hundreds of years. Methods of control include frightening by the use of noise-making apparatus, or the use of alarm calls of the particular species involved. These methods, require persistence and must sometimes be reinforced by shooting. Structures and other objects can sometimes be protected by the use of tacky physical repellents or by the use of netting or other barriers which deny access to roosting space. Unprotected pest species may be captured in traps or nets or, if baits are accepted, destroyed by stomach poisons.

Bats, together with their ectoparasites, can be controlled by heavily treating their roosts with 50% DDT water-dispersible powder. The powder may be dusted on to previously moistened roosts or it may be sprayed using a thin slurry containing 1 lb of 50% water-dispersible powder per gallon of water. Bat-proofing, consisting of the closure of all cracks larger than 60 mm, may be practical; if so, it should be completed while the bats are

out of the structure, i.e., in winter in northern climates, or in the evening after all bats have left the roost.

14. PROTECTION AGAINST EXPOSURE TO PESTICIDES

The following information and recommendations are reproduced, with slight modifications, from the twelfth report of the Expert Committee on Insecticides (Toxic Hazards of Pesticides to Man).[1] They are intended as a guide to those responsible for the use of pesticides in public health programmes of vector control.

14.1 Protection of operators

After years of use with the minimum of safety precautions and absence of any evidence of poisoning, DDT was replaced in some programmes by dieldrin without any realization of the fact that the known mammalian toxicity of dieldrin made it probable that its use under such conditions would present a greater hazard to operators if they handled it in the same way as they had handled DDT. Casualties from dieldrin poisoning—fortunately with few, if any, fatalities—have been reported from almost every programme in which the material has been applied as an indoor spray for any length of time. Precautions have been suggested by a Study Group on the Toxic Hazards of Pesticides to Man,[2] but casualties have occurred even where these precautions were observed and the discipline of the spray teams was good.

These cases of poisoning among spray operators have led to a greater realization of the hazards to which these men may be exposed in vector-control programmes. Because of the limitations that must apply to the application of the findings in studies of acute and chronic toxicity on laboratory animals, it is now realized that exposure to a potentially hazardous material must be first undertaken in carefully supervised programmes in which a special watch is kept on the health of the spray operators.

Following the recommendation made in the report of the above-mentioned Study Group, work has been carried out to assess the nature of the hazards to which indoor spraymen applying water-dispersible-powder suspensions are exposed.[3] These studies show that vastly more of the deposit falls on the body surface than is inhaled, but the amounts falling on the skin itself will naturally depend upon the degree to which this is protected. Light cotton cloth provides an adequate protection from water-dispersible-powder suspensions.

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[1] *Wld Hlth Org. techn. Rep. Ser.*, 1962, **227**.

[2] *Wld Hlth Org. techn. Rep. Ser.*, 1956, **114**.

[3] Wolfe, H. R., Walker, K. C., Elliott, J. W. & Durham, W. F. (1959) *Bull. Wld Hlth Org.*, **20**, 1.

The amount of pesticide absorbed by the dermal route will depend not only upon the area of exposed skin but also upon the readiness with which the particular pesticide penetrates the skin. The inhalation hazard will depend mainly on the size of the particles; with wettable powders it is found that by the time they reach the face of the operator they are virtually dry and are too large to penetrate to the depth of the lungs. Material of this nature, after entering the nose, is ultimately swallowed. Further experiments showed that substantial protection of both the airways and the exposed skin of the face could be achieved by wearing a light veil held away from the face on a broad-brimmed hat. Vision through the veil is not obstructed by spray deposit as it is with a plastic visor.

Absolute protection of the skin and respiratory tract imposes physical limitations that would make house-spraying in hot climates impossible. It is therefore necessary to ensure that the precautions recommended are adequate to meet the likely hazard and that their exercise will allow the work to be carried out effectively. The strangeness of the attire of a suitably protected operator should not be allowed to interfere with the spraying programmes merely because the protected population does not appreciate its significance. In this context the importance of public health education must be stressed. It is encouraging to note that certain protective equipment is acceptable because its possession adds to the worker's comfort and to his prestige.

14.1.1 *General aspects of hazard*

Apart from the risks involved in the handling of bulk shipments—risks to which spray operators will not normally be exposed—hazards may arise (*a*) in transferring pesticide at headquarters stations from bulk packages to smaller containers, and in carrying it to the site of spray operations; (*b*) in the preparation of spray concentrates; (*c*) from continued exposure to droplets in the course of spraying; (*d*) from accidental contamination at any stage.

14.1.2 *General precautions for all spray operators applying pesticides*

All pesticides are toxic to some degree, and care in handling all types should be routine practice. The following *minimum precautions* are recommended for operators handling pesticides in public health work:

(1) All operators handling pesticides should be informed of the risks involved in their use and receive instructions for handling them safely.

(2) There should be adequate technical and medical supervision of operators, together with the provision of facilities for the treatment of any casualties. Supplies of atropine, some in special syringes ready for immediate injection and some in tablet form, should be available in all

first-aid kits when anticholinesterase insecticides are being applied. Atropine should not be issued to spraymen for self-medication, but the supervisor of the field operations should be trained to administer atropine in emergencies.

(3) Operators applying pesticides of all types should wear some form of impervious head covering, which should be regularly cleaned. A broad-brimmed hat with a veil together with an impervious cape to cover the shoulders should be worn in all programmes where new pesticides are being applied, since they may be appreciably more hazardous to use than DDT or malathion.

(4) Operators who have scratches on the skin or skin irritation at places likely to be exposed to the insecticide should not be permitted to work, since such damage is known to facilitate the penetration of the pesticides into the body.

(5) Facilities, including soap, should be provided for washing the skin and clothing. Compulsory washing after the daily work should be a supervised routine in all spraying operations.

(6) Operators should not be exposed to a pesticide for more than four to five hours during a normal working day if they are required to apply that pesticide day after day over long periods.

(7) Separate working clothes should be used; they should be changed and washed as frequently as possible. An occasional rinse in kerosene will effectively remove deposits of chlorinated hydrocarbon pesticides not taken out by soap and water. Ordinary washing soda is a better decontaminant for the organophosphorus insecticides than any of the modern detergents in use.

(8) Workers should not smoke during their work nor eat without first washing their hands, and they should take other simple hygienic precautions in places where pesticides are handled.

(9) Since, in all pesticide work, the greatest hazard lies in the handling of concentrates, it is recommended that, in the transferring of concentrates from drums, either threaded taps or drum-pumps of standard design be employed. Furthermore, attention is drawn to the following recommendations made in the sixth report of the Expert Committee on Insecticides: [1]

" In preparing concentrations of water-dispersible powders, use must be made of deep mixing-vessels and long-handled mixers to protect the operator from splashing and to permit stirring from a standing position.

" For the dilution of solid pastes, electrically operated or power appliances are satisfactory and permit the dilution to be prepared in a closed

[1] *Wld Hlth Org. techn. Rep. Ser.*, 1956, **110**, 21.

vessel. Where such appliances are not available, long-handled mixers and tall vessels should be provided. No vessel shall be filled to a point where the operator will be endangered through splashing. Operators employed in the mixing of concentrates should, in addition to the protective clothing and devices recommended [for spraymen], be provided, with impermeable aprons. Long-handled dippers or scoops should be used for transferring the insecticide from one vessel to another...

" Liquid concentrates should be supplied in strong cans, not easily damaged in transportation, tightly sealed to avoid any leakage, but with a cover that may be easily removed.

" For transportation of the liquid concentrates, use may be made of military-type tanks provided with pressure caps and a small aspiration tube so as to avoid a sudden flow when drawing off. "

(10) Equipment used in spraying should conform to the general and specific recommendations with regard to design and maintenance included in *Specifications for pesticides*.[1]

(11) All pesticide containers should be adequately labelled to identify the contents and show, *in a form comprehensible to the operator*, the nature of the material and the precautions to be employed.

(12) It is important to ensure the safe disposal of empty or nearly empty containers. They must not be allowed to go astray or be removed by unauthorized people, who may be tempted to use them as containers of food or drinking water, especially in areas where such containers are scarce.

The following procedure is known to be effective in decontamination of used containers, provided that they have not been allowed to become rusty.

The container is rinsed two or three times with water and the sides scrubbed. If it has contained an organophosphorus compound an additional rinse should be carried out with 5% washing soda and the solution allowed to remain in the container overnight. Rubber gauntlets should be worn during this work, and a soakage pit should be provided for the rinsings.

14.1.3 *Clinical surveillance of operators*

A sprayman should always have ready access to a physician to ensure early detection of any symptoms indicative of acute over-exposure to the pesticides or harmful effects of long-term exposure. Apart from clinical examination, quantitative biochemical tests may be carried out in an attempt to assess the degree of exposure.

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[1] World Health Organization (1961) *Specifications for pesticides*, 2nd ed., Geneva.

The significance and importance of regular determination of blood cholinesterase activity where organophosphorus compounds are used are discussed in section 14.4.4. Equipment for carrying out this test in the field is available, and methods may be further improved as experience is gained.

In view of individual differences and the lack of knowledge of the average levels of activity of the blood cholinesterase in populations living in different parts of the world,[1] it is essential that pre-exposure levels be determined on all operators, and some attempt should also be made to assess any fluctuations occurring during the observation period in unexposed members of the population.

Detailed procedures have been described for measuring the exposure of spray operators.[2]

The recommendations given above demand the provision of certain facilities ; it is important that the responsibility for providing these and for taking care of the health of spray operators should be clearly delineated before a spray programme is started.

With the increased awareness of the possible hazards, it is likely that the care of spray operators will form an important part of programmes involving the application of pesticides until such time as it is possible to use chemicals that present no hazard to those who handle them.

14.2 Third parties

Accidents to children and others often arise from inadequate attention to the safe custody of pesticide concentrates. Those in charge of programmes using pesticides must ensure that suitably qualified people take full responsibility for the custody of stocks and the disposal or treatment of empty or half-empty containers. The diversion of pesticides for personal or domestic use has led to a number of accidents.

14.3 Signs and symptoms of anticholinesterase poisoning [3]

(*a*) *Following local exposure*

Site of action	*Signs and symptoms*
Pupils	Miosis, marked, usually maximal (pin-point), sometimes unequal
Ciliary body	Frontal headache, eye pain on focusing, slight dimness of vision, occasional nausea and vomiting
Conjunctivae	Hyperaemia

[1] Barnes, J. M., Hayes, W. J. & Kay, K. (1957) *Bull. Wld Hlth Org.*, **16**, 41.

[2] Durham, W. F. & Wolfe, H. R. (1962) *Bull. Wld Hlth Org.*, **26**, 75.

[3] After Holmstedt, B. (1959) *Pharmacol. Rev.*, **11**, 567.

Site of action	*Signs and symptoms*
Nasal mucous membranes	Rhinorrhoea, hyperaemia
Bronchial tree	Tightness in chest, sometimes with prolonged wheezing expiration suggestive of bronchoconstriction or increased secretion, cough
Sweat glands	Sweating at site of exposure to the liquid
Striated muscle	Fasciculations at site of exposure to the liquid

(*b*) *Following systemic absorption*

Site of action	*Signs and symptoms*
Bronchial tree	Tightness in chest, with prolonged wheezing expiration suggestive of bronchoconstriction or increased secretion, dyspnoea, slight pain in chest, increased bronchial secretion, cough
Gastro-intestinal system	Anorexia, nausea, vomiting, abdominal cramps, epigastric and substernal tightness (? cardiospasm) with "heartburn" and eructation, diarrhoea, tenesmus, involuntary defecation
Sweat glands	Increased sweating
Salivary glands	Increased salivation
Lachrymal glands	Increased lachrymation
Heart	Slight bradycardia
Pupils	Slight miosis (occasionally unequal), later more marked
Ciliary body	Blurring of vision
Bladder	Frequent or involuntary urination
Striated muscle	Easy fatigue, mild weakness, muscular twitching, fasciculations, cramps, generalized weakness including muscles of respiration, with dyspnoea and cyanosis
Sympathetic ganglia	Pallor, occasional elevation of blood pressure
Central nervous system	Giddiness ; tension ; anxiety ; jitteriness ; restlessness ; emotional lability ; excessive dreaming ; insomnia ; nightmares ; headache ; tremor ; apathy ; withdrawal and depression ; bursts of slow waves of elevated voltage in EEG especially on overventilation ; drowsiness ; difficulty in concentrating ; slowness of recall ; confusion ; slurred speech ; ataxia ; generalized weakness ; coma with absence of reflexes ; Cheyne-Stokes respiration ; convulsions ; depression of respiratory and circulatory centres with dyspnoea, cyanosis, and fall in blood pressure

14.4 Organophosphorus compounds and carbamates

14.4.1 *Mechanism of action*

The effects of the organophosphorus compounds and carbamates in question have been related to the inhibition of blood and tissue cholinesterases and thus to an accumulation of excessive amounts of acetylcholine in effector organs.

TABLE 9. MAMMALIAN TOXICITIES OF SOME ORGANOPHOSPHORUS INSECTICIDES

Insecticide	LD_{50} (mg per kg of body weight)								Maximum tolerated concn. in diet [a] (p.p.m.)
	Single oral dose						Single skin application		
	Rat (M)	Rat (F)	Mouse	Guinea-pig	Rabbit	Hen	Rat	Rabbit	Rat
Parathion	12.5	3.5	25.0	32.0		5 [b]	21	40	25
Systox	10	4				20 [b]		24	20
Dichlorvos	80	56					75		1000
Diazinon	150		80	320	130	25 [b]		> 4000	1000
Fenthion	215	615	150	> 800 [b]	150 [b]	40 [b]	500 (M & F)		250
Malathion	1375	1000	3000	570		500 [b]	> 4400		1000

[a] Concentration tolerated without gross effects.
[b] Approximate figure.

The compound may itself bring about this enzyme inhibition. Dichlorvos is an example of such a " direct inhibitor ". In many instances the compound has first to be metabolized in the body and converted into the inhibitor; malathion and fenthion are examples of these "indirect inhibitors ".

The symptoms of intoxication are mostly, if not exclusively, of a cholinergic nature. Some correlation exists between the rate and degree of inhibition of blood cholinesterase and the severity of the symptoms. This correlation is not strict under all conditions of intoxication. Other mechanisms of action cannot be excluded as the cause of some of the toxic effects.

The duration of symptoms of poisoning depends in part on the rate of reactivation of the inhibited cholinesterase and the rate at which the inhibitor itself is destroyed or removed from the tissues. Both these factors are related to the chemical structure of the compound.

The mammalian toxicities of some organophosphorus insecticides are shown in Table 9.

14.4.2 *Effects*

The effects of anticholinesterases have been reviewed at length by Holmstedt.[1] They are listed in section 14.3, page 210 and a few notes are added here.

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[1] Holmstedt, B. (1959) *Pharmacol. Rev.*, **11**, 567.

(*a*) *Local effects*

Miosis is most commonly seen after the eye has been exposed to the vapour or particles of a direct inhibitor. Direct inhibitors may sometimes be present as impurities in commercial preparations of indirect inhibitors. Miosis may be unilateral if particles enter one eye only. It may be accompanied by lachrymation, a feeling of pressure in the eye, conjunctival hyperaemia, spasm of accommodation, and dim vision.

Salivation, nasal discharge and hyperaemia with excessive bronchial secretion and bronchospasm will result from the exposure of the upper and lower portions of the respiratory tract to direct inhibitors.

If these substances are ingested, gastro-intestinal spasm with colic, hypersecretion, vomiting and diarrhoea are common early symptoms.

(*b*) *Systemic effects*

These are in general similar whatever the route of absorption, but differences in their sequence and time course may occur.

(*c*) *Delayed effects*

A few organophosphorus compounds, such as mipafox, (but none in current use as pesticides) have been shown to possess a delayed neurotoxic action.

14.4.3 *Causes of death in anticholinesterase poisoning*

As causes of death in poisoning by cholinesterase inhibitors, the following main factors have been shown to be operative :

Respiratory system : increased secretion, bronchospasm, neuromuscular block of respiratory muscles, paralysis of respiratory centre, asphyxia.

Circulatory system : bradycardia, decreased cardiac output, cardiac arrest, paralysis of vasomotor centre.

Central nervous system : convulsions.

Death appears to be primarily asphyxial in some instances and cardiovascular in others.

14.4.4 *Diagnosis of intoxication*

Diagnosis of anticholinesterase poisoning can be made from a history of exposure followed by the onset of all or some of the signs and symptoms listed in section 14.3, page 210. After exposure to the indirect type of inhibitor, the first symptoms may not appear until after the operator has left work. In some instances, they may develop during the night and their association with occupational poisoning may not be immediately recognized.

The diagnosis can be confirmed by determination of the activity of the blood cholinesterases. This, however, may not be depressed in cases where local symptoms follow exposure to direct inhibitors, as would be brought about by a splash of dichlorvos concentrate on the face and eyes.

The determination of the activity of the blood cholinesterases is of even greater value as an index of exposure, because it is possible to have depression of activity in the complete absence of symptoms.

Several methods are available for determining cholinesterase activity.[1] Separate determination of the plasma and red-cell cholinesterase gives the most accurate information. Whole-blood tests may be easier to perform in the field but are less sensitive and subject to greater experimental errors, making it difficult to detect small fluctuations in activity. There is an advantage in determining the activity of plasma cholinesterase alone, because few organophosphorus compounds are known which do not depress activity in the plasma before that in the red cells.

Because the Tintometer method developed by Edson [2] is practical under field conditions and experience in its use has been gained, this method should continue to be used at the present time.

The activity of blood cholinesterases should be determined regularly in operators exposed to the organophosphorus compounds of intermediate or greater toxicity. Operators should be withdrawn from exposure if the activity of their blood cholinesterase decreases by 25% or more from a well-established pre-exposure value.

The insecticidal carbamates give rise to a more rapidly reversible cholinesterase-inhibitor complex. This makes it impossible to use estimates of cholinesterase activity *in vitro* as an accurate index of the activity of this enzyme in the tissues.

14.4.5 *Therapy of poisoning by cholinesterase inhibitors*

The correct treatment of anticholinesterase poisoning includes—in addition to the use of drugs—removal of the toxic agent and decontamination of exposed skin with an alkaline solution or soap and water. Suction of the airways, tracheotomy, and positive-pressure artificial respiration may be necessary at a later stage. These measures have been dealt with extensively in various reviews.[3] It must be stressed that all drug treatment is liable to fail if the other measures are not properly observed. Drugs used for this purpose fall into several groups as follows :

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[1] Augustinsson, K. B. (1957) Assay methods for cholinesterase. In : *Methods in biochemical analysis*, London, Interscience ; Barnes, J. M., Hayes, W. J. & Kay, K. (1957) *Bull. Wld Hlth Org.*, **16**, 41.

[2] Tintometer Ltd. (1956) The rapid field determination of cholinesterase in whole blood. In : *Colorimetric chemical analytical methods*, Salisbury, England.

[3] Holmstedt, B. (1959) *Pharmacol. Rev.*, **11**, 567.

(*a*) *Atropine*

Atropine sulfate is recommended as first aid in the case of poisoning with organophosphorus compounds or carbamates.

Atropine should be injected immediately after the appearance of any local pulmonary or systemic signs of anticholinesterase poisoning, in doses somewhat larger than those employed for other purposes.

When signs or symptoms produced by an anticholinesterase compound are *mild*, 1-2 mg of atropine should be injected intramuscularly and the injection repeated, if necessary, at 30-minute intervals until symptoms are relieved. Any patient sick enough to receive even one dose of atropine should be under medical observation for at least 24 hours, because the symptoms may reappear.

When symptoms of poisoning are *moderately severe*, 2-4 mg of atropine should be injected intravenously or, if this is not feasible, intramuscularly. Repeated doses of 2 mg at 10-minute intervals should be given until symptoms are relieved.

When *severe* symptoms are present (particularly respiratory difficulties, marked slowing of the heart or convulsions), 4-6 mg of atropine should be injected intravenously or, if this is not feasible, intramuscularly, and repeated doses of 2 mg should be injected at 3- to 8-minute intervals until the severe symptoms decrease markedly or cease. In severe anticholinesterase poisoning the effect of each injection of atropine may be transient, lasting only 10-30 minutes. The patient must therefore be observed as closely as possible for recurrence of signs of poisoning, and atropine must be repeated at appropriate intervals for at least 48 hours if his clinical condition requires it. In severe poisoning, as much as 24-28 mg of atropine may be required the first day. Respiratory failure requires immediate artificial respiration.

Attention ought to be given to the fact that treatment with atropine may reduce heat loss, owing to inhibition of sweating. Under hot environmental conditions this may be dangerous, particularly in young children. It is therefore advisable that the hospitalized patient be kept cool and quiet. Under no circumstances must a person who has received even one dose of atropine be allowed to perform muscular work, and he must be kept under observation for at least 24 hours or until he is fully recovered.

Devices, including automatic injectors of reliable construction, are now available for the administration of atropine or other drugs of similar solubility and stability. It is recommended that a supply of these injectors should be at hand during spraying operations but should not be issued to the individual spraymen.

(*b*) *Alternatives and supplements to atropine*

In addition to the oximes (see (*c*) below), substitutes for or supplements to atropine include atropine analogues, anticonvulsants such as Tridione,

and a series of compounds derived from benactyzine that also have antidotal action.[1] Parsidol and Diparcol have also been suggested.

Although some atropine analogues may be superior to atropine for treating poisoning by specific compounds, none has been found that is better in all respects.

(*c*) *Reactivators (oximes)*

In recent years it has been demonstrated that certain oximes, when used in combination with atropine, provide more effective therapy than atropine alone in poisoning with some anticholinesterases.[2] These drugs reactivate phosphorylated cholinesterase and thus tend to correct the biochemical lesion rather than merely relieve symptoms. Of the oximes, 2-pyridinium aldoxime methiodide (2-PAM-iodide) has been used in man in accidental poisoning by organophosphorus compounds. The intravenous administration of 2000 mg of 2-PAM-iodide to man at the rate of 100-300 mg/min. produces no signs or symptoms and no change in blood pressure or cardiac rate. The safety of the compound is shown by the fact that repeated doses totalling over 40 g have been given intravenously without side-effects in a case of severe poisoning.

Because of its low solubility in water (5% w/v), 2-PAM-iodide can be administered intravenously only. *N*-methylpyridinium-2-aldoxime methane sulfonate (P2S) (70% w/v) and 2-pyridinium aldoxime methochloride (2-PAM-chloride) (100% w/v) are much more soluble. The manufacture and distribution of these more soluble salts should be encouraged.

It has been demonstrated that the results obtained with an oxime in relation to a particular organophosphorus compound are not necessarily applicable to other organophosphorus compounds, even those closely related chemically. The potential usefulness of the 2-PAM salts and other reactivators should, therefore, be investigated when new insecticides of the organophosphorus type are introduced.

After poisoning by some organophosphorus compounds, the intravenous administration of 500-2000 mg of 2-PAM-iodide counteracts generalized weakness to a moderate degree, reduces muscular fasciculations and relieves neuromuscular block. Improvement may begin within 30 seconds after injection of oxime and become maximal in 5-10 minutes. Approximately 20 minutes later there may be some return of weakness and fasciculations, but not to the original level. A second injection of oxime then results in further improvement. There is some reversal of the inhibition of blood cholinesterase. In parathion poisoning treated by 2-PAM-iodide, the reversal is confined largely to the red-cell cholinesterase and, in some cases, is permanent after a single dose. Since respiratory weakness

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[1] Anichov, S. S. (1961) *Ann. Rev. Pharmacol.*, **1**, 21.

[2] Holmstedt, B. (1959) *Pharmacol. Rev.*, **11**, 567 (Table 4).

may develop rapidly in the course of severe anticholinesterase intoxication, mechanical measures to sustain respiration are still needed for some patients.

It must be stressed that the 2-PAM salts and other reactivators are of limited value if not accompanied by other therapeutic measures such as atropine and artificial respiration. It is recommended that 2-PAM-iodide, 2-PAM-chloride, P2S or a similar compound be included in the kits of medically trained toxicologists who accompany spraying teams using organophosphorus insecticides.

In severe intoxication by an organophosphorus compound, 1000 mg of one of the 2-PAM analogues should be slowly injected intravenously after the previous administration of atropine. The dose may be repeated after 20 minutes if weakness is not relieved or if it recurs. In moderate intoxication half the above dose should be used and similarly repeated.

The following drugs must *not* be given to people poisoned with anticholinesterases: morphine, barbiturates, tranquillizers.

15. PREPARATION AND APPLICATION OF FORMULATIONS

The following recommendations relate to the use of sprays of solutions, emulsions, water-dispersible powders and dusts. An emulsion is a two-phase system having a reasonable degree of stability in which a liquid is dispersed in small droplets within a second liquid. Emulsions are formed by diluting an emulsion concentrate in water. An emulsion concentrate is a single-phase liquid system containing an insecticide together with one or more surface-active agents having the property of forming an emulsion on dilution with water. In a water-dispersible powder the toxicant is incorporated in a finely-powdered inert carrier together with suitable wetting and spreading agents so that it will form a suspension when added to water.

The following precautions should be observed while applying insecticides:

(1) Food, eating utensils, animal feed, feed troughs, drinking fountains, and milk or food-processing equipment should not be contaminated.

(2) Pesticides and their formulations must be kept in a safe place where children, pets and other animals cannot reach them.

(3) Oil solutions should not be sprayed near open flames, electric coils, or electric power outlets.

(4) Before applying residual sprays near electric wires or fuse boxes, the power should be turned off.

(5) Operators should not smoke or strike matches while applying pesticides.

The information given in the following tables will enable operators to prepare formulations accurately and to convert the recommended percentage concentrations of spray and quantities to be applied per given area into dosages in g per m^2 or mg per ft^2 or *vice versa*.[1]

TABLE 10. AMOUNT OF FINISHED SPRAY OF VARYING CONCENTRATIONS REQUIRED TO PRODUCE SPECIFIC DOSAGES OF TOXICANT PER UNIT OF SURFACE AREA

Concentration of technical insecticide (%)	Litres (US gal) of spray required per 100 m^2 (1000 ft^2) to give approximately:			
	2 g/m^2 (200 mg/ft^2)	1 g/m^2 (100 mg/ft^2)	0.5 g/m^2 (50 mg/ft^2)	0.2 g/m^2 (20 mg/ft^2)
5	4 (1)	2 (0.5)	1 (0.25)	0.4 (0.1)
2.5	8 (2)	4 (1)	2 (0.5)	0.8 (0.2)
1	20 (5)	10 (2.5)	5 (1.25)	2 (0.5)
0.5	40 (10)	20 (5)	10 (2.5)	4 (1)
0.25	80 (20)	40 (10)	20 (5)	8 (2)

TABLE 11. DILUTION FACTOR FOR EMULSION CONCENTRATES

Concentration of emulsion concentrate (%)	Parts of water to be added to 1 part of emulsion concentrate when concentration of final formulation is:					
	0.25%	0.5%	1%	2.5%	5%	10%
50	199	99	49	19	9	4
25	99	49	24	9	4	1.5
10	39	19	9	3	1	—

The general formula is:

$$X = \frac{A}{B} - 1$$

in which X = parts of water to be added to 1 part of emulsion concentrate
A = concentration of the emulsion concentrate (%)
B = required concentration of the final formulation (%)

Example: A 0.5% formulation is to be prepared from a 25% concentrate:

$$X = \frac{25}{0.5} - 1 = 49$$

49 parts of water to 1 part of concentrate are required.

[1] One mg per ft^2 is equivalent to 10.76 mg per m^2; conversely 1 g per m^2 is equivalent to 92.9 mg per ft^2. For practical purposes, however, the conversion rate used in this report is: 100 mg per ft^2 = 1 g per m^2.

TABLE 12. AMOUNT OF WATER-DISPERSIBLE POWDER REQUIRED FOR PREPARATION OF FINISHED SPRAY SUSPENSIONS

Concentration of water-dispersible powder (%)	Kg (lb) of water-dispersible powder required for about 380 litres (100 US gal ; 83 Imp gal) of finished spray suspension at percentage concentrations of:				
	0.25%	0.5%	1%	2.5%	5%
75	1.25 (2.8)	2.5 (5.6)	5.0 (11.1)	12.6 (27.8)	25.2 (55.6)
50	1.9 (4.2)	3.8 (8.3)	7.6 (16.7)	18.9 (41.7)	37.8 (83.3)
25	3.8 (8.3)	7.6 (16.7)	15.1 (33.3)	37.8 (83.3)	75.6 (166.7)

The general formula is :

$$X = \frac{A \times B \times D}{C}$$

in which X = amount of water-dispersible powder required
A = percentage concentration desired
B = quantity of spray desired
C = percentage concentration of water-dispersible powder
D = 1 if X and B are expressed in kg and litres, respectively
D = 8.33 if X and B are expressed in lb and US gal, respectively
D = 10 if X and B are expressed in lb and Imp gal, respectively

Example : 100 US gal of 1 % spray suspension are to be prepared from 50% powder

$$X = \frac{1 \times 100 \times 8.33}{50} = 16.7$$

16.7 lb of water-dispersible powder are required.

TABLE 13. AMOUNT OF TECHNICAL MATERIAL REQUIRED FOR PREPARATION OF EMULSION CONCENTRATES

Concentration desired (%)	Kg (lb) of technical material per 3.8 litres (1 US gal) of concentrate	Amounts of materials to make about 190 litres (50 US gal ; 41.5 Imp gal) of concentrate		
		Technical material	Emulsifier [a]	Solvent
35	1.32 (2.92)	66.2 kg (146 lb)	3.8 litres (1 US gal) (0.8 Imp gal)	Enough to make volume to 190 litres
25	0.94 (2.08)	47.2 kg (104 lb)	,,	(50 US gal) (41.5 Imp gal)
15	0.57 (1.25)	28.3 kg (62.5 lb)	,,	
12.5	0.47 (1.04)	23.6 kg (52 lb)	,,	
6.25	0.24 (0.52)	11.8 kg (26 lb)	,,	

[a] 2% of the concentrate.

The general formula is :

$$X = \frac{A \times B \times C}{100}$$

in which X = amount of technical material required
A = percentage concentration desired
B = quantity of emulsion concentrate desired
C = { 1 if X and B are expressed in kg and litres, respectively
8.33 if X and B are expressed in lb and US gal, respectively
10 if X and B are expressed in lb and Imp gal, respectively

Example : 50 US gal of 25% concentrate are to be prepared :

$$X = \frac{25 \times 50 \times 8.33}{100} = 104$$

104 lb of technical material are required.

TABLE 14. PARTS PER MILLION BY WEIGHT OR VOLUME

1 p.p.m. = 1 mg (0.015 grain) per kg
= 1 g (15.4 grains) per metric ton
= 0.007 grain (0.45 mg) per lb
= 1 ml (0.035 Imp fl oz) per m^3
= 0.16 Imp fl oz (4.546 ml) per 1000 Imp gal
= 0.128 US fl oz (3.785 ml) per 1000 US gal

TABLE 15. PARTS PER MILLION EQUIVALENTS USING A 25% EMULSION CONCENTRATE

p.p.m.	Amount of 25% concentrate [a] needed per 3.8 million litres of water (1 million US gal ; 0.83 million Imp gal)		Amount of toxicant per 3.8 million litres of water (1 million US gal ; 0.83 million Imp gal)	
1	4 US gal 3.3 Imp gal	15.1 litres	8.3 lb	3.8 kg
0.1	3.2 US pt 2.65 Imp pt	1.51 litres	13.33 oz	380 g
0.01	5.12 US fl oz 5.3 Imp fl oz	151 ml	1.33 oz (583 grains)	38 g
0.001	0.5 US fl oz 0.5 Imp fl oz	15.1 ml	0.13 oz (58 grains)	3.8 g

[a] Containing 0.94 kg (2.08 lb) of toxicant per 3.8 litres (1 US gal ; 0.83 Imp gal).

TABLE 16. AMOUNTS OF % DUST OR % CONCENTRATE REQUIRED FOR SPECIFIC DOSAGES PER UNIT OF SURFACE AREA

Dosage		Amount of 25% concentrate required		Amount of 5% dust required	
lb	kg			lb	kg
10	4.54	4.8 US gal 4.0 Imp gal	18.2 litres	200	90.7
5	2.27	2.4 US gal 2.0 Imp gal	9.1 litres	100	45.4
3	1.36	1.4 US gal 1.2 Imp gal	5.5 litres	60	27.2
2.2	1.0	1.1 US gal 0.9 Imp gal	4.2 litres	44	20.0
1	0.45	1.9 US qt 1.6 Imp qt	1.8 litres	20	9.1
0.5	0.23	1.9 US pt 1.6 Imp pt	900 ml	10	4.5
0.1	0.045	6.1 US fl oz 6.4 Imp fl oz	200 ml	—	—

The general formulae are:

$$\text{for concentrates} \quad X = \frac{A \times 100}{B \times C}$$

$$\text{for dusts} \quad X = \frac{A \times 100}{B}$$

in which X = amount of concentrate (gal) or dust (lb) required
A = dosage per acre (lb)
B = percentage concentration of the product used
C = 8.33 if X is expressed in US gal
10 if X is expressed in Imp gal

Examples: For a dosage of 10 (lb) per acre,

(*a*) using a 25% concentrate, $X = \frac{10 \times 100}{25 \times 8.33} = 4.8$

4.8 US gal of concentrate are required per acre;

(*b*) using a 5% dust, $X = \frac{10 \times 100}{5} = 200$

200 lb of dust are required per acre.

TABLE 17. EQUIVALENCE OF GRAMS PER HECTARE, POUNDS PER ACRE, AND p.p.m. IN WATER OF DEPTHS [a] OF 1 AND 12 INCHES

Treatment dosage		p.p.m. at depth	
g/ha	lb/acre	1 in	1 ft
2 240	2.0	8.8	0.74
1 120	1.0	4.4	0.37
560	0.5	2.2	0.18
280	0.25	1.1	0.092
112	0.10	0.44	0.037
56	0.05	0.22	0.018
28	0.025	0.11	0.0092
11	0.01	0.044	0.0037

[a] The p.p.m. at other depths or other treatment dosages can be obtained by simple proportion ; for example, the p.p.m. at depths of 2, 4, 8 and 16 in or ft are $^1/_2$, $^1/_4$, $^1/_8$ and $^1/_{16}$ of those at 1 in or 1 ft, respectively.

TABLE 18. NUMBER OF ACRES [a] IN VARIOUS SIZE AREAS

Length of area	Number of acres in areas with indicated width (ft)					
	30	40	50	100	200	300
1 mile	3.60	4.80	6.00	12.00	24.00	36.00
660 ft ($^1/_4$ mile)	0.45	0.60	0.75	1.50	3.00	4.50
500 ft	0.34	0.46	0.57	1.15	2.30	3.45
300 ft	0.21	0.28	0.35	0.69	1.38	2.07
200 ft	0.14	0.18	0.23	0.46	0.92	1.38
100 ft	0.07	0.09	0.12	0.23	0.46	0.69

[a] Other values can be determined by simple proportion or by the formula :
Acres = 0.121 × (width in ft) × (length in miles)
Hectares = 100 × (width in km) × (length in km) = 10 × (width in m) × (length in km)

16. CONVERSION FACTORS : METRIC AND ENGLISH UNITS

Length

1600 m	= 1.6 km	= *1 mile*	= 1760 yd = 5280 ft
1×10^5 cm	= 1000 m	= *1 kilometre* (km)	= 0.625 mile = 1100 yd
91.4 cm	= 0.91 m	= *1 yard* (yd)	= 3 ft = 36 in
1000 mm	= 100 cm	= *1 metre* (m)	= 1093 yd = 328 ft = 39.37 in
0.3048 m	= 30.48 cm	= *1 foot* (ft)	= 12 in
25.4 mm	= 2.54 cm	= *1 inch* (in)	= 1/12 ft

Length *(continued)*

10 000 μ	= 10 mm	= *1 centimetre* (cm)	= 0.394 in = 0.033 ft
	1000 μ	= *1 millimetre* (mm)	= 0.0394 in
0.001 mm	= 0.0001 cm	= *1 micron* (μ)	= 0.000039 in (about 1/25 000 in)

Area

	259 ha	= *1 square mile* (sq mile)	= 640 acres
	100 ha	= *1 square kilometre* (km^2)	= 0.39 sq mile = 247 acres
10 000 m^2	= 0.01 km^2	= *1 hectare* (ha)	= 2.47 acres
4 047 m^2	= 0.405 ha	= *1 acre*	= 4840 yd^2 = 43 560 ft^2
	10 000 cm^2	= *1 square metre* (m^2)	= 1.2 yd^2 = 10.76 ft^2 = 1550 in^2
	0.84 m^2	= *1 square yard* (yd^2)	= 9 ft^2 = 1296 in^2
930 cm^2	= 0.093 m^2	= *1 square foot* (ft^2)	= 144 in^2
	6.45 cm^2	= *1 square inch* (in^2)	= 0.007 ft^2
	100 mm^2	= *1 square centimetre* (cm^2)	= 0.155 in^2
	93 m^2	= *1000 square feet* (ft^2)	

Volume

1000 litres	= *1 cubic metre* (m^3)	= 1.307 yd^3 = 35.32 ft^3
2.83 m^3	= *100 cubic feet* (ft^3)	= 3.7 yd^3
0.77 m^3	= *1 cubic yard* (yd^3)	= 27 ft^3
28.32 litres	= *1 cubic foot* (ft^3)	= 0.037 yd^3 = 1728 in^3
16.39 cm^3	= *1 cubic inch* (in^3)	= 0.000579 ft^3

Liquid capacity

3.79 litres	= *1 US gallon* (US gal)	= 0.83 Imp gal = 231 in^3
4.55 litres	= *1 Imperial gallon* (Imp gal)	= 1.2 US gal
1000 ml	= *1 litre*	= 0.26 US gal = 0.22 Imp gal
32 fluid ounces	= *1 quart* (qt)	= 2 pints (pt) = 0.25 gal

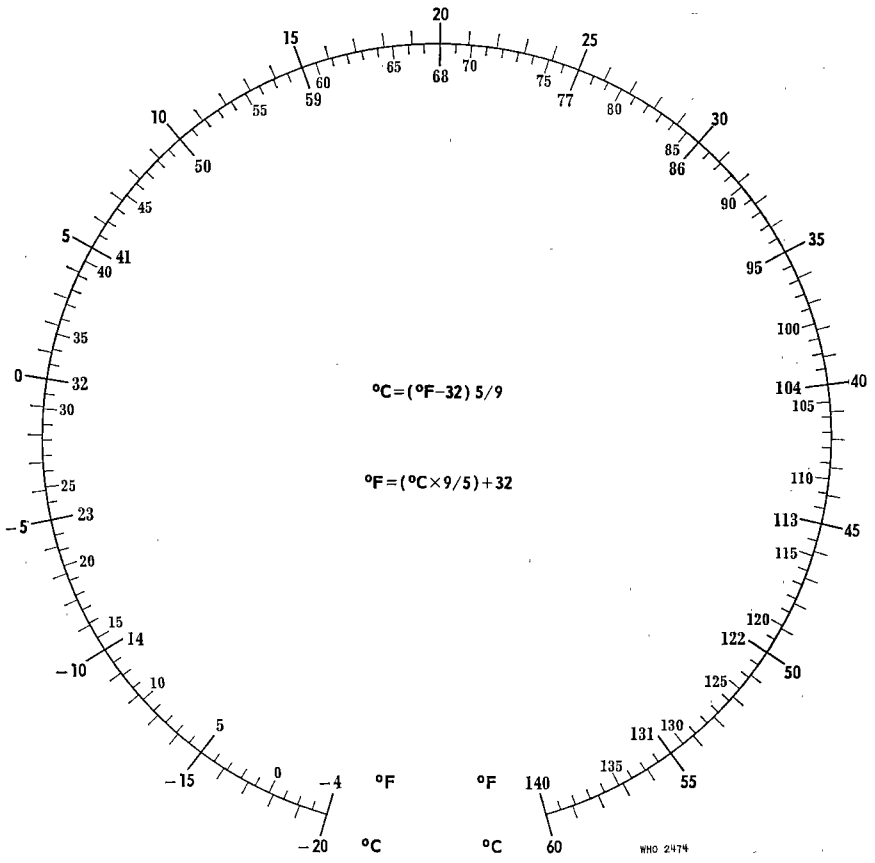
Weight

1000 mg	= *1 gram* (g)	= 0.0352 oz
28.35 g	= *1 ounce* (oz)	= 1/16 lb = 437.5 grain
64.8 mg	= *1 grain*	= 1/7000 lb
453.6 g	= *1 pound* (lb)	= 16 oz
1000 g	= *1 kilogram* (kg)	= 2.2 lbs = 35.27 oz
1000 kg	= *1 metric ton*	= 2200 lb
907 kg	= *1 US short ton*	= 2000 lb = 0.893 US long tons
1018 kg	= *1 US long ton*	= 2240 lb = 1 Br. ton = 1.12 US short ton

Weight of water in various volumes at 16.7°C (62°F)

1 ft^3	= 62.3 lb
1 litre	= 1000 g, 1 kg, 2.2 lb
1 US gal	= 8.3 lb
1 Imp gal	= 10 lb

TEMPERATURE CONVERSION



17. LIST OF COMMON AND CHEMICAL NAMES OF PESTICIDES

Pesticide	*Chemical Name*	*Other Names*
allethrin [1]	(±)-2-allyl-4-hydroxy-3-methylcyclopent-2-en-1-one, esterified with a mixture of cis- and trans-(±)-chrysanthemum monocarboxylic acid	
aldrin [1]	a product containing 95% HHDN (q.v.)	
antu [1]	1-naphthylthiourea	
Baytex	see fenthion	
BHC [1]	mixed isomers of 1,2,3,4,5,6-hexachlorocyclohexane	HCH

[1] Name recommended by the International Organization for Standardization (ISO)

Pesticide	*Chemical Name*	*Other Names*
chlordane [1]	1,2,4,5,6,7,10,10-octachloro-4,7,8, 9-tetrahydro-4,7-methyleneindane	chlordan, Velsicol 1068, Octa-Klor, Octachlor
Chlorthion	*O,O*-dimethyl *O*-(3-chloro-4-nitrophenyl)phosphorothioate	Bayer 22/190
coumachlor [1]	3-(α-acetonyl-4-chlorobenzyl)-4-hydroxycoumarin	
DDD	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane	Rhothane, TDE
demeton-*O* [1]	*O,O*-diethyl *O*-[2-(ethylthio)ethyl] phosphorothioate	mercaptofos
demeton-S [1]	*O,O*-diethyl *S*-[2-(ethylthio)ethyl-] phosphorothioate	mercaptofos teolovy
diazinon [1]	*O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidyl)phosphorothioate	
Dibrom	1,2-dibromo-2,2-dichloroethyl dimethyl phosphate	naled
dicapthon	*O*-(2-chloro-4-nitrophenyl) *O,O*-dimethyl phosphorothioate	Am. Cyanamid 4124 Di-Captan
dichlorvos [1]	2,2-dichlorovinyl dimethyl phosphate	DDVP, Nuvan, Vapona
dieldrin [1]	a product containing 85% HEOD (q.v.)	
dimetilan	1-dimethylcarbamoyl-5-methyl-3-pyrazolyl dimethylcarbamate	Dimetilane 65-13332
dimethoate	*S*-methylcarbamoylmethyl *O,O*-dimethyl phosphorodithioate	Rogor, Am. Cyanamid 12880
diphacinone	2-diphenylacetylindane-1,3-dione	diphacin
Dipterex	see trichlorfon	
DDT	1,1,1-trichloro-2,2-di(*p*-chlorophenyl) ethane	
DDVP	see dichlorvos	
EPN	*O*-ethyl *O*-*p*-nitrophenyl phenylphosphorothioate	
fenthion [1]	*O,O*-dimethyl-*O*-(3-methyl-4-methylmercaptophenyl) phosphorothioate	Baytex, Entex, Bayer 29493
formalin	37% aqueous solution of formaldehyde	
Fumarin	3-((+)-furyl-β-acetylethyl)-4-hydroxycoumarin	coumafuryl
HEOD [1]	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-endo-1,4-exo-5,8-dimethanonaphthalene	

[1] Name recommended by the International Organization for Standardization (ISO)

Pesticide	*Chemical Name*	*Other Names*
heptachlor [1]	1,4,5,6,7,10,10-heptachloro-4,7,8, 9-tetrahydro-4,7-methyleneindene	Velsicol 104
HHDN [1]	1,2,3,4,10,10-hexachloro-1,4,4a,5, 8,8a-hexahydro-endo-1,4-exo-5,8-dimethanonaphthalene	
isopropyl cresol	mixed isomers of mono- and di-isopropyl cresols	Phenol-S
Kepone	decachlorooctahydro-1,3,4-metheno-2 *H*-cyclobuta[*cd*]pentalen-2-one	
lindane [1]	a product containing not less than 99% of the gamma isomer of BHC	gamma-BHC gamma-HCH
malathion [1]	*S*-[1,2-bis(ethoxycarbonyl)ethyl] *O*,*O*-dimethyl phosphorodithioate	carbofos, malathon, compound 4049
methoxychlor [1]	1,1,1-trichloro-2,2-di-(4-methoxyphenyl)ethane	Marlate, DMDT, methoxy DDT
methyl bromide	bromomethane	
methyl parathion	see parathion-methyl	
paradichlorobenzene	1,4-dichlorobenzene	
parathion [1]	*O*,*O*-diethyl *O*-(4-nitrophenyl) phosphorothioate	Thiophos, Niran
parathion-methyl [1]	*O*,*O*-dimethyl *O*-(4-nitrophenyl) phosphorothioate	
Perthane	1,1-dichloro-2,2-bis(*p*-ethylphenyl) ethane	
pindone [1]	2-pivaloylindane-1,3-dione	pival
piperonyl butoxide	(3,4-methylenedioxy-6-propylbenzyl)(butyl)diethylene-glycol ether	
pival	see pindone	
pyrethrum extract	extract from dried pyrethrum flowers	
Pyrolan	3-methyl-1-phenyl-5-pyrazolyl dimethylcarbamate	
ronnel	*O*,*O*-dimethyl *O*-(2,4,5-trichlorophenyl)phosphorothioate	Trolene, Korlan, Nankor Viozene, fenchlorfos
rotenone	the poisonous principle of derris rhizome and root	derris root
Sevin	1-naphthyl-*N*-methylcarbamate	
sodium monofluoroacetate	sodium salt of 2-fluoroacetic acid	1080
sucrose	sucrose	
sulfoxide	1,2-methylenedioxy-4-(2-octylsulfinylpropyl)-benzene	

[1] Name recommended by the International Organization for Standardization (ISO)

Pesticide	*Chemical Name*	*Other Names*
Systox	mixture of demeton-O and demeton-S	
toxaphene	chlorinated camphene	
trichlorfon [1]	dimethyl 2,2,2-trichloro-1-hydroxy-ethylphosphonate	Dipterex, Bayer L 13/59, chlorophos, Nevugon
warfarin [1]	3-(α-acetonylbenzyl)-4-hydroxy-coumarin	

[1] Name recommended by the International Organization for Standardization (ISO)

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